SEP 2 2 1943

Official Organ of the Society for the Study of Development and Growth

GROWTH

A JOURNAL FOR STUDIES OF DEVELOPMENT AND INCREASE

PERIODICAL ROOM
GENERAL LIBRARY
UNIV. OF MICH.

VOLUME VII, NUMBER 3
PAPERS 235 - 242

SEPTEMBER, 1943 **$5.00 A YEAR**

Entered as second class matter on October 20, 1941, at the Post Office, Worcester, Mass., under the Act of March 3, 1879.

GROWTH

A journal for the coördination of studies of increase and development as general properties of nature. Published by contributors and subscribers on a coöperative, non-profit, non-salary basis. The field of investigation is primary and work will be published in which the approach is through mathematics, physics, chemistry, genetics, embryology, philosophy, and anthropology; just as will studies of development in its several expressions, and of mass increase and the part played therein by nutrition, vitamines, and hormones.

The journal will appear regularly at quarterly intervals. Subscription is $5.00 per volume (outside U. S. and Canada, $5.50). Single numbers $2.00. There will be one volume of at least 400 pages each year.

Subscriptions should be sent to *The Editors of Growth,* either at *150 Fremont St., Worcester, Mass.,* or at *Lankenau Hospital Research Institute, Girard and Corinthian Avenues, Philadelphia, Pa.* All manuscripts should be sent to *The Editors of Growth, Lankenau Hospital Research Institute, Girard and Corinthian Avenues, Philadelphia, Pa.*

THE EDITORS OF GROWTH

John Berrill, McGill University; S. Brody, University of Missouri; H. S. Burr, Yale University; S. A. Courtis, University of Michigan; C. H. Danforth, Stanford University; Chas. B. Davenport, Carnegie Institute; L. K. Frank, Macy Foundation; P. W. Gregory, University of California; F. S. Hammett, Lankenau Hospital; Leigh Hoadley, Harvard University; Clyde Kluckhohn, Harvard University; Warren H. Lewis, The Wistar Institute; Leo Loeb, Washington University; Carroll E. Palmer, U. S. Public Health Service; Otto Rahn, Cornell University; H. S. Reed, University of California; S. P. Reimann, Lankenau Hospital; R. E. Scammon, University of Minnesota; E. W. Sinnott, Yale University; K. V. Thimann, Harvard University; Paul Weiss, University of Chicago; Philip R. White, Rockefeller Institute; D. M. Whitaker, Stanford University; B. H. Willier, Johns Hopkins University; Sewall Wright, University of Chicago.

Corresponding Editors

B. Boysen Jensen, University of Copenhagen; Ivar Broman, Tornblad Institutet; Luigi Castaldi, Università di Cagliari; A. Dalcq, Université de Bruxelles; E. Fauré-Fremiet, College de France; Julian S. Huxley, Zoölogical Society; J. Krizenecky, Vysoka Skola Zemedelska; Joseph Needham, Caius College; Yo. K. Okada, Kyoto Imperial University; G. Teissier, Faculté des Sciences; A. H. Roffo, Universidad de Buenos Aires.

Growth, 1943, **7**, 257-264.

THE CHEMICAL GROWTH OF THE BRAIN OF THE WHITE RAT AND ITS RELATION TO SEX

ARTHUR WEIL

Institute of Neurology, Northwestern University Medical School, Chicago, Illinois
(Received for publication on April 10, 1943)

In reviewing the literature on the chemical development of the brain of both man and laboratory animals, one is surprised to find that hardly any attention is paid to sexual differences. Most of the voluminous literature is concerned with lipid analysis (Page, '37) without regard to sex, and much of this earlier work has to be discarded because formalin fixed material was used (Weil, '29). Apparently most of the authors assumed a priori that there are no qualitative differences in the chemical constitution of the male and female brain and that the chemical development of both follows the same pattern though with quantitative differences.

In a previous paper (Weil, '41), it has been demonstrated that there are definite qualitative differences between the phospholipids of the brains of male and female white rats and also between male and female human brains (Weil and Liebert, '43). No attempt, however, had been made in the first paper to determine the relative distribution of the phosphorus in the different phospholipid fractions of the rat brain. Furthermore, rats 155 to 183 days old were used and the figures which were published were the mean values of these different age groups combined. Following the reasoning of previous authors it had been assumed that with maturity there should be no essential difference in the qualitative chemical composition of the brain of the two sexes. That this working hypothesis is not justified will be demonstrated in the present paper. Furthermore, it will be shown that the diet plays an important rôle in determining the chemical make-up of the brain of the white rat, at least during the first three months of life.

In one series of experiments the rats were fed on the following Diet 1: white bread, 4 lbs.; bread crumbs, 1 lb.; milk, 1.25 quarts; dog chow (canned, dry), 1 lb.; beef hearts (ground), 1 lb.; carrots

once a week; lettuce twice a week. This diet was calculated for about 100 rats of an average weight of 200 grams. In a second group of experiments the rats were fed a Sherman Diet 2, which has been described in a previous paper (Weil, '37). The body weight of corresponding age groups between 100 and 180 days and which were fed on Diet 2 was about 30 per cent less for the males and 26 per cent less for the female rats if compared with animals fed on Diet 1.

The technique was the same as that which had been employed before (Weil, '41). The brains from animals of the same litter and sex were pooled. Altogether 19 different litters were analyzed comprising 64 male and 52 female rats fed with Diet 1. The different lipid fractions were isolated and their phosphorus content was analyzed. In addition the phosphorus content of the alcoholic extract only, was determined in 50 male and 23 female rats which had been raised on Diet 2.

Briefly summarized the technique was as follows: Immediately after removal of the brain from the decapitated animal, it was cut into small pieces and kept for about 24 hours in 20 times its volume of acetone. The latter dehydrates the brain and dissolves the neutral fats, fatty acids, cholesterol and its esters. Next, the brain is dried at a temperature not exceeding 50° C. and extracted with boiling alcohol in the Soxhlet apparatus for four hours. The alcoholic extract is evaporated to a volume of about 20 cc. and then poured into 10 times its volume of acetone. The precipitate which is formed is subjected to fractionate extraction with alcohol, ether, and pyridine at room temperature. The alcohol will contain mainly the lecithins, the ether cephalin, the pyridine galactolipids, and the residue consists mainly of sphyngomyelin. The Fisk-Subarrow method was used for the phosphorus determinations.

The influence of the diet upon the growth of the brain is demonstrated in Figure 1. The growth of the brain of man (Roessle and Roulet, '32) is compared with the growth of the rat brain calculated from Donaldson's ('15) figures (Curve 2, male and female) and of the rats fed on Diet 1 (Curve 1). Donaldson calculated the relative life span between man and rat as 30:1 (l.c.) and this relative proportion of the ages was used in constructing the two sets of curves in Figure 1. These curves indicate that the maximal brain weight is reached in the white rat at an age of approximately 7 to 8 months and in man at about 20 to 25 years of age. Diet 1 brought about a more

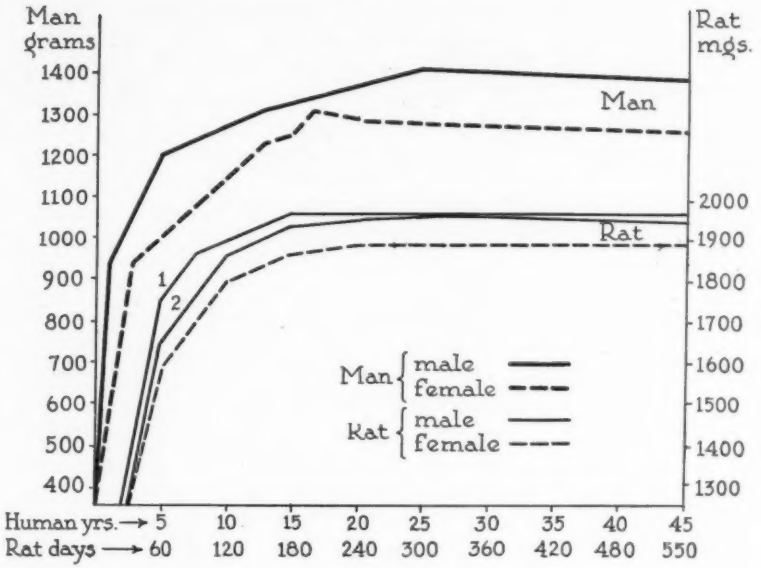

FIGURE 1

COMPARISON OF THE WEIGHT OF MALE AND FEMALE HUMAN AND RAT BRAINS AT DIFFERENT AGES

Rat Curve 1 corresponds to the brain weight of male rats fed on Diet 1 (cf. text); Curve 2 corresponds to the brain weight of male rats of the Wistar Institute fed on table scrap (Donaldson, '15). Figures for the human brain from Roessle and Roulet ('32).

rapid growth of the brain than did the diet of the Wistar Institute (table scrap).

The chemical changes which take place during growth in the phospholipid content of the brain of the two sexes in rats are graphically illustrated in Figure 2. The graphs demonstrate the differences between the phospholipid content of the brains of the two sexes regardless of the diet and thus confirm the more generalized conclusions of the preceding paper (Weil, '41). The figure also shows that the male brain becomes chemically mature at an earlier age than that of the female rat brain; the curve breaks abruptly at about 75 days (Diet 1), an age which is approximately coincident with the onset of puberty in the rat and with the age of about six years in man, if we accept Donald-

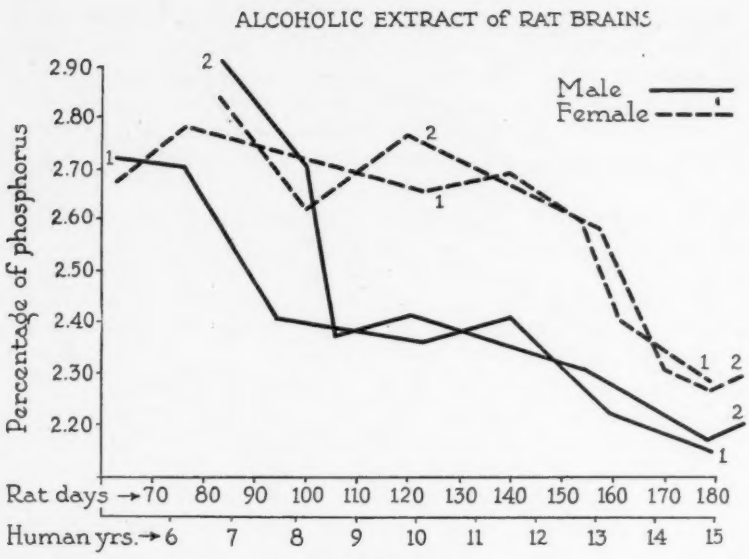

FIGURE 2

PHOSPHORUS CONTENT OF THE ALCOHOLIC BRAIN EXTRACT IN PERCENTAGE OF THE EXTRACT

Male and female Curves 1 refer to rats fed on Diet 1 (cf. text); Curves 2 refer to rats fed on Diet 2. For comparison human years have been superimposed upon the rat days, assuming a relative proportion of human age to rat age of 30:1 (Donaldson, '32).

son's ('15) ratio of 1:30. The latter age in man is coincident (Figure 1) with a sudden change in the growth curve of the brain. The curve of the female rat breaks somewhat less abruptly at about 140 days (Figure 2) and the phosphorus content of the alcoholic extract stays on a level of about 2.70 per cent, up to this age, as compared with 2.40 per cent of the male rat brain at the age of 140 days. The two curves gradually approach each other, until at the age of six months the difference between male and female brain becomes less than .15.

Figure 2 illustrates, furthermore, the influence of different diets upon the chemical differentiation of the brain. The male brain shows the sudden break of the curve in rats fed with Diet 2 about one month later than in the male rats which were fed on Diet 1. The curve for the females, also, remains on a higher phosphorus level for a longer period in the rats fed with Diet 2 than in those fed with Diet 1.

In the following tables and figures the data have been condensed into the three age groups of (*a*) 65 to 80 days, (*b*) 90 to 130 days, (*c*) 140 to 180 days. This grouping coincides approximately with the sudden changes of the curve of Figure 2 in both male and female brains. All the figures are mean values obtained from a combination of analyses of brains within the same age group.

Table 1 demonstrates that the water content of both the male and

TABLE 1

RATS FED ON DIET 1; WEIGHT OF BODY AND BRAIN; FRACTIONAL EXTRACTION OF BRAINS WITH ACETONE AND ALCOHOL

Age group days	Body weight grams		Brain weight mgs		Water per cent		Acet. extr. % of dry		Alcoh. extr. % of dry		Residue % of dry	
	M	F	M	F	M	F	M	F	M	F	M	F
65- 80	236	173	1765	1700	78.1	78.9	15.3	14.7	30.8	30.0	53.9	55.3
90-130	352	204	1920	1780	77.3	77.8	16.0	16.3	31.4	30.7	52.6	53.0
140-180	430	234	1973	1810	76.9	76.8	15.2	15.5	35.0	34.5	49.8	50.0

female brain decreases with age, while the relative amount of alcoholic extract is increased; but the difference between the two sexes at a given age group is very small. The difference between the male and female brain is the more impressive, therefore, if one compares the phosphorus content of the alcoholic extracts from the brain of animals older than three months (Table 2). The same applies to a lesser degree to the

TABLE 2

PHOSPHORUS CONTENT OF DIFFERENT FRACTIONS OF EXTRACTION; PHOSPHORUS IN PERCENTAGE OF FRACTIONS

Age group days	Acetone extract		Alcohol extract		Residue		Total phosphorus % of dry weight	
	M	F	M	F	M	F	M	F
65- 80	1.41	1.44	2.71	2.76	.91	.93	1.54	1.54
90-130	1.44	1.50	2.42	2.72	.92	.94	1.51	1.56
140-180	1.46	1.45	2.40	2.54	.95	.97	1.53	1.56

non-extractable phosphorus of the residue of extraction. The female values are constantly greater than the male values.

The next problem was to analyze the alcoholic extracts in order to determine the amount of different phospholipids. MacLean's method was used for this purpose as described in previous publications ('41). The figures which were obtained from an analysis of the acetone precipitates of the alcoholic extracts again demonstrate that the results

which had been published in the first publication cannot be applied to younger rats because they had been obtained from the combined precipitates of rat brains 155 to 183 days old.

Several interesting points are brought out by the study of Table 3.

TABLE 3

FRACTIONAL EXTRACTION OF THE ACETONE PRECIPITATE OBTAINED FROM THE ALCOHOLIC EXTRACT; PERCENTAGES OF EXTRACTS

Age group	Alcoh. sol.		Ether soluble		Pyridine sol.		Residue	
	M	F	M	F	M	F	M	F
65- 80	13.5	13.5	70.4	72.6	7.2	8.9	8.9	5.0
90-130	16.9	15.2	66.3	72.4	8.5	5.7	8.3	6.7
140-180	16.3	15.6	55.3	63.4	13.8	9.4	14.6	11.6

First, in all of the age groups the female brain contains a relatively larger amount of ether-soluble phospholipids (cephalin) than the male brain. Second, the amount of alcohol-soluble (lecithins), pyridine-

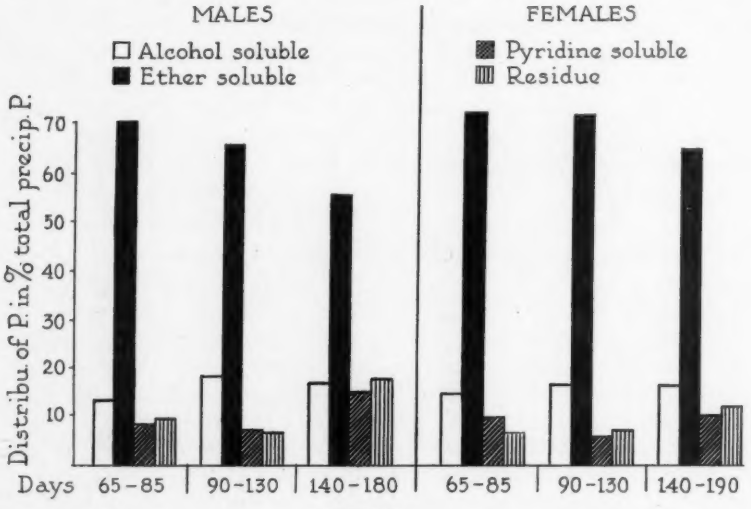

FIGURE 3

THIS FIGURE DEMONSTRATES THE DISTRIBUTION OF THE LIPID PHOSPHORUS AMONG THE FOUR FRACTIONS PREPARED FROM THE ACETONE PRECIPITATE OF THE ALCOHOLIC EXTRACT It shows that relatively more phosphorus is contained in the cephalin fraction of the female brain in comparison with the male brain. It furthermore demonstrates the decrease of cephalin phosphorus with age in both sexes.

soluble (galactolipids) and-insoluble lipids (sphyngomyelins) is larger for the male brains. This difference is graphically illustrated in Figure 3, in which the percentage distribution of the precipitate phosphorus between the different fractions has been plotted for the two sexes and the three different age groups (cf. also Table 4).

TABLE 4

PHOSPHORUS CONTENT OF THE TOTAL PRECIPITATE AND OF ITS SEPARATE FRACTIONS IN PERCENTAGES

Age group days	Total precipitate		Alcohol sol.		Ether sol.		Pyridine sol.		Residue	
	M	F	M	F	M	F	M	F	M	F
65- 80	2.80	2.69	2.50	2.39	3.45	3.05	1.63	1.79	1.60	1.90
90-130	2.43	2.60	2.50	2.68	2.67	2.76	1.82	2.06	1.71	1.82
140-180	2.27	2.38	2.12	2.25	2.60	2.64	1.62	1.72	2.21	2.06

If one analyzes the data in Table 4, one will find a qualitative difference between the phospholipids of the more mature animals, 90 to 180 days old. The values for the females are constantly higher than those for the males. It must be left to further studies and to a refinement of the methods of isolation of phospholipids to decide whether this difference is due to a greater admixture of non-phosphorus containing lipids in the male brain, or whether the phospholipids of the female rat brain are structurally different from the male ones.

SUMMARY AND CONCLUSIONS

1. The amount of ether-soluble phospholipids (cephalin) is greater in the female rat brain than in the male brain after puberty. With progressing age it is gradually replaced in both by proportionally greater amounts of alcohol-soluble (lecithins), pyridine-soluble (galactolipids), and-insoluble lipids (sphyngomyelins).

2. Higher values for the phosphorus content of the alcoholic extract and of the different lipid fractions in the female brain represent a second difference between the male and female brain.

3. The male brain matures chemically earlier than the female brain. There is a sudden diminution in the amount of phosphorus of the alcoholic extract at the age of about three months, while in the female brain this change is delayed up to about the fifth month of life.

4. When rats are fed a laboratory diet richer in calories and more abundant in natural food stuffs than a standard Sherman diet, this

chemical maturation of the brain takes place in the male about one month earlier than in the female.

REFERENCES

1. DONALDSON, H. H. 1915. The Rat. Philadelphia: Memoirs of the Wistar Institute of Anatomy and Biology, No. 6. Pp. 278.
2. MACLEAN, H., & I. MACLEAN. 1927. Lecithin and Allied Substances. New York, Longmans, Green. Pp. 220.
3. PAGE, I. H. 1937. Chemistry of the Brain. Springfield, Ill.: Thomas. Pp. 444.
4. ROESSLE, R., & F. ROULET. 1932. Mass und Zahl in der Pathologie. Berlin, Springer. Pp. 144.
5. WEIL, A. 1929. The influence of formalin fixation on the lipoids of the central nervous system. *J. Biol. Chem.*, **83**, 601-609.
6. ———. 1937. The phospholipids of the brain, kidneys, and heart of white rats in experimental hyperthyroidism. *Endocrinology*, **31**, 101-109.
7. ———. 1941. The correlation between sex and chemical composition of the central nervous system of white rats. *Endocrinology*, **29**, 150-154.
8. WEIL, A., & E. LIEBERT. 1943. The correlation between sex and chemical constitution of the human brain. *Quarterly Bulletin, Northwestern Univ. Med. School*, **17**, 117-120.

Growth, 1943, **7**, 265-271.

ENDOCRINE GLAND WEIGHTS OF CHICK EMBRYOS*

W. G. VENZKE

Department of Veterinary Anatomy, Iowa State College, Ames, Iowa
(Received for publication on April 20, 1943)

Recent investigations of the relation of the endocrine glands to growth, in the chick and in the adult fowl, have led to a similar study for the chick embryo. After a review of the literature it is evident that no complete data on the endocrine gland weights of chick embryos are really available.

The growth in weight of the various endocrine glands for the White Leghorn chicken has been studied by Latimer (1924); for the adult Bantam, by Leonard and Righter (1936); for the Frizzle fowl, by Landauer and Aberle (1935); for Single-comb White Leghorn and Rhode Island Red chicks from hatching until 30 days of age, by Breneman (1941); and for the adult Brown Leghorn, by Juhn and Mitchell (1929).

Aberle and Landauer (1935) determined the actual and relative thyroid weight from newly hatched chicks resulting from matings of White Leghorns, homozygous Frizzles, and reciprocal crosses between these two breeds. Sun (1932) reported the weights of the thyroid and adrenal glands from the ninth day of incubation until hatching.

MATERIAL AND METHODS

The data presented in this investigation are concerned with possible sex differences in the weights of the hypophysis, thymus, thyroids, adrenals, ovaries, and testes of Single-comb White Leghorn chick embryos. The fertile eggs were obtained from a group of highly bred hens maintained by the Iowa State College Department of Poultry Husbandry. The eggs were incubated in an electrically operated Buffalo incubator at a temperature ranging between 101° and 103° F. The relative humidity was not determined, since Penquite (1938)

*A portion of the data presented in a thesis submitted to the graduate faculty of Iowa State College in partial fulfilment of the requirements for the degree, Doctor of Philosophy, March, 1942.

showed that the percentages of relative humidity did not cause a significant difference in the growth of the embryo as measured by wet weight.

A series of 240 embryos was used for determining the weights and rate of growth of the endocrine glands. The glands of 10 male and 10 female embryos were weighed each 24 hours from the 10th through the 21st day of incubation. The embryonic yolk sac and membranes were removed and the wet weights of the embryos in the fresh state were recorded. Each gland, or pair of glands, was carefully removed, freed of all vessels, nerves, and adjacent connective tissue, and kept in a moist chamber until weighed. Immediately following their removal from the embryo, all glands were weighed on a chemical balance sensitive to 0.1 milligram. The results show the growth rate in weight of the endocrine glands for both sexes as a percentage of the total body weight.

Results

1. *Embryo Weights*

The data on the body weights of both the male and female embryos are summarized in Tables 1 and 2. The figures show that growth rate is most rapid in both sexes between 11 and 12 days of incubation, and that the female embryos are rather consistently heavier than the males. The average coefficient of variability is $11.81 \pm .09$ for the males and $10.64 \pm .08$ for the females.

2. *Hypophysis*

In Tables 1 and 2 it is interesting to note that there is a slow but gradual decrease in the percentage values for both sexes from the 10th to the 21st day of incubation. If there is a difference between the weights of the embryonic hypophyses of the male and female embryos, it is slightly in favor of a heavier male hypophysis. This statement is based on the fact that at no time during the incubation period are the hypophyses of the female heavier than those of the male embryos when corrected for body weight. The average coefficient of variability for the males is 15.26 ± 2.41, and 15.64 ± 3.00 for the females.

3. *Thymus*

The thymus of the chick embryo follows the usual course of devel-

TABLE 1

MEAN BODY WEIGHTS AND PERCENTAGE WEIGHTS OF THE ENDOCRINE GLANDS OF FEMALE CHICK EMBRYOS

Age in days	No. of embryos	Body weight gms.	Hypophysis mgs.	Thymus mgs.	Thyroids mgs.	Adrenals mgs.	Ovaries mgs. Right	Ovaries mgs. Left
10	10	2.01± .07	.014±.002	.042±.005	.015±.003	.040±.007	.013±.004	.036±.009
11	10	2.75± .11	.013±.002	.058±.003	.015±.003	.047±.019	.010±.003	.033±.004
12	10	4.52± .20	.011±.002	.091±.013	.015±.002	.034±.011	.008±.002	.045±.012
13	10	6.42± .28	.013±.003	.130±.007	.019±.006	.033±.009	.009±.004	.046±.013
14	10	9.35± .23	.010±.003	.137±.004	.015±.006	.026±.005	.003±.002	.042±.009
15	10	12.80± .59	.007±.002	.159±.011	.018±.003	.028±.005	.004±.004	.036±.007
16	10	16.25± .48	.009±.001	.211±.015	.024±.007	.031±.006	.002±.0008	.033±.011
17	10	20.00± .39	.007±.001	.236±.010	.025±.006	.025±.003	.003±.001	.039±.012
18	10	23.40± .52	.006±.0005	.247±.011	.025±.004	.023±.003	.004±.002	.034±.003
19	10	23.60± .91	.006±.0004	.254±.024	.026±.002	.024±.003	.003±.001	.033±.005
20	10	31.58± .73	.004±.0003	.321±.017	.022±.004	.022±.004	.002±.001	.025±.003
21	10	33.75±1.19	.004±.0003	.291±.019	.021±.005	.024±.004	.002±.0006	.024±.004

TABLE 2

MEAN BODY WEIGHTS AND PERCENTAGE WEIGHTS OF THE ENDOCRINE GLANDS OF MALE CHICK EMBRYOS

Age in days	No. of embryos	Body weight gms.	Hypophysis mgs.	Thymus mgs.	Thyroids mgs.	Adrenals mgs.	Testes mgs.
10	10	2.26± .05	.014±.004	.052±.005	.014±.004	.035±.009	.025±.007
11	10	2.61± .12	.014±.002	.061±.006	.016±.003	.042±.013	.026±.006
12	10	4.34± .22	.012±.003	.098±.011	.015±.006	.035±.007	.017±.004
13	10	6.29± .23	.014±.002	.133±.012	.019±.007	.032±.007	.019±.007
14	10	8.76± .37	.010±.002	.158±.010	.014±.003	.024±.006	.017±.004
15	10	12.67± .56	.008±.003	.163±.007	.021±.006	.027±.006	.028±.006
16	10	15.85± .58	.009±.001	.239±.025	.025±.006	.028±.005	.034±.006
17	10	18.60± .50	.008±.002	.269±.022	.022±.003	.027±.003	.034±.006
18	10	20.36± .99	.007±.0009	.291±.016	.022±.004	.026±.004	.031±.003
19	10	23.75± .76	.006±.0007	.353±.014	.026±.003	.028±.005	.030±.008
20	10	31.29± .68	.005±.0004	.332±.020	.024±.002	.024±.004	.023±.001
21	10	33.70±1.34	.004±.0003	.310±.019	.022±.003	.023±.003	.022±.002

opment in that there is a progressive increase in the percentage of weight in both sexes from the 10th day of incubation until just before hatching. As shown in Tables 1 and 2, the percentage values are consistently less for the female as compared with the male embryos.

The average coefficient of variability for the thymi of the males is 26.02 ± 2.37, and 27.86 ± 3.68 for the females. Some of this variability is the result of difficulty in getting the thymic lobes dissected free of fascia and fat. However, it is a fact that the thymic tissue weights vary considerably between individual embryos even though they have the same body weight, age, and sex.

4. *Thyroid*

The thyroid glands of both sexes develop slowly but gradually. The percentage gland weights show that the thyroids of both sexes are heaviest at 19 days of incubation. Determined on a percentage of weight basis, no sex differences occur in the thyroid glands. If the weight of the gland is indicative of its activity, it might be concluded that the thyroid glands of both sexes are least active from 16 to 21 days (Tables 1 and 2). The average coefficient of variability for the males is 20.95 ± 2.04 and 25.28 ± 4.52 for the females.

5. *Adrenal*

There is a gradual decrease in the percentage weights of the adrenals in both sexes from the 11th to the 21st day. However, based on percentage of weight, the adrenals do not consistently show sex differences (Tables 1 and 2). The average coefficient of variability is 22.68 ± 2.89 for the males, and 21.75 ± 2.67 for the females.

6. *Ovary*

When the left ovary is considered as a percentage of the body weight, the largest figures occur between the 12th and 14th day; the smallest occur between the 20th and 21st day. Some of the percentage figures for the latter fourth of the incubation period are large and may be the result of sampling error (Table 1). The average coefficient of variability for the left ovary is 21.34 ± 2.10.

The percentage weights of the right ovary decrease from the 10th to the 21st day (Table 1). The right ovaries are so small that much

difficulty is experienced in dissecting them out, and as a result, the average coefficient of variability is 44.64 ± 5.36.

7. *Testis*

Considering the testes as percentages of the total body weight, they are smallest between the 12th and 14th day. From the 17th day until hatching there is a gradual decrease in the percentage weight (Table 2). In general the testes are variable between individual embryos of the same group. The average coefficient of variability is 24.38 ± 2.87.

Discussion

Latimer (1924) has presented data on the postnatal growth of the Single-comb White Leghorn fowl hypophyses, in which he found no appreciable sex difference when the weights were plotted against body weight. If there were a difference, it was in favor of a heavier male hypophysis.

If the weight of a gland is indicative of its functional activity, it might be concluded that the hypophysis is most active at the close of the incubation period, and least active at 10 to 11 days. Some evidence to defend the statement that the weight and the activity of a gland are associated, is presented by Juhn and Mitchell (1929). These workers found that caponization induced a hypertrophy of the hypophysis with a significant increase in the percentage weight of the gland as compared with that of normal males. This increase in weight is undoubtedly produced by the storage of the gonadotropic hormone in the pars glandularis.

It is interesting to note that there is a rather abrupt decrease in the thymi weights of both sexes just at hatching. The percentage decrease is not a permanent one in view of the data presented by Latimer (1924). He stated that the thymus percentage weight rises from a little less than 0.3 per cent of the net body weight to 0.46 per cent at 110 days. After this time there is a decrease to approximately 0.05 per cent for adult chickens.

Leonard and Righter (1936) found no significant sex difference in the weights of the thyroids in the Bantam. Juhn and Mitchell (1929) observed that the thyroids of the Brown Leghorn are heavier in the male than in the female birds. However, Aberle and Landauer (1935) recalculated the relative thyroid weight from the original data pre-

sented by Juhn and Mitchell and found that the difference amounted to only twice the standard error. Aberle and Landauer (1935) found a sex difference which is statistically significant for the thyroid weight in newly hatched chicks resulting from matings of White Leghorns, homozygous Frizzles, and reciprocal crosses between these two breeds. Latimer (1924) obtained little evidence indicative of a sex difference in the thyroid weight of the growing Single-comb White Leghorn. Breneman (1941) observed that during the first 30 postnatal days the thyroid glands of both sexes decrease in relative size with age, but that this is much more marked in the pullets.

Sun (1932) presented data showing the weight per pair of thyroid glands from Leghorn chick embryos. The glands from both sexes were grouped together, and the mean weights from the 9th to the 20¼th day were presented. As a whole, the thyroid gland weights given in Sun's data are consistently less than those presented in Tables 1 and 2.

Juhn and Mitchell (1929) observed that in adult Brown Leghorn chickens no pronounced sex differences are present in the weights of the adrenals. Latimer (1924), working with Single-comb White Leghorn chickens, observed that the adrenal glands are somewhat variable in weight but show no sex differences. Breneman (1941) found in White Leghorn chicks from the 5th to the 30th day after hatching that the adrenal glands are of a uniform proportion throughout the series, becoming lighter in relative weight at the 30th day. Leonard and Righter (1936) found no sex difference in size of the adrenals of the Bantam fowl.

Sun (1932) presented data for the weight per pair of adrenal glands from the 9½ through the 20th day of incubation. The gland weights were determined for both sexes as a group, and they compare favorably with those reported in this investigation.

Breneman (1941) has data to show that the female gonad increases in weight from the 5th day after hatching until the 20th day, at which time the figures begin to plateau. Latimer (1924) observed that in Leghorn chickens the ovaries are extremely variable in weight.

Breneman (1941) obtained data to show that a definite increase in size takes place in the testes from the 5th to the 15th day of postnatal life; at which time they reach their maximum size in relation to body weight. Latimer (1924) observed that the testes are quite variable in the adult Leghorn chicken.

Summary and Conclusions

1. A series of weights was obtained of the hypophysis, thymus, thyroids, adrenals, ovaries, and testes from Single-comb White Leghorn chick embryos from the 10th to the 21st day of incubation.

2. The data show that the body weight increases most rapidly in both sexes between 11 and 12 days of incubation, and that the female embryos are slightly heavier than the males.

3. The weights of the right ovary, thymus, and thyroids from the females, and the testes are most variable.

4. In the hypophysis no sex differences could be ascertained on a percentage of weight basis other than that the female glands are consistently lighter.

5. Ascertained on a percentage of weight basis, no sex differences exist in the thymi other than that the female glands are consistently lighter.

6. Determined on a percentage of weight basis, no sex differences occur in the thyroid glands from the 10th to the 21st day of incubation.

7. Based on percentage of weight, the adrenal glands do not consistently show sex differences.

8. Based on percentage of weight the right ovary decreases from the 10th until the 21st day.

9. Based on percentage of weight the weight of the left ovary decreases slowly from the 13th day until hatching.

References

1. Aberle, S. D., & W. Landauer. 1935. Thyroid weight and sex in newly hatched chicks. *Anat. Rec.*, **62**, 331-335.
2. Breneman, W. R. 1941. Growth of the endocrine glands and viscera in the chick. *Endocrinology*, **28**, 946-954.
3. Juhn, M., & J. B. Mitchell, Jr. 1929. On endocrine weights in Brown Leghorns. *Am. J. Physiol.*, **88**, 177-182.
4. Landauer, W., & S. D. Aberle. 1935. Studies on the endocrine glands of the Frizzle fowl. *Am. J. Anat.*, **57**, 99-134.
5. Latimer, H. B. 1924. Postnatal growth of the body, systems, and organs of the Single-comb White Leghorn chicken. *J. Agr. Res.*, **29**, 363-397.
6. Leonard, S. L., & J. W. Richter. 1936. Endocrine weights of the Bantam fowl. *J. Hered.*, **27**, 363-366.
7. Penquite, R. 1938. Influence of temperature and humidity upon the growth of chick embryos in a mechanically ventilated incubator. *Iowa Agr. Exp. Sta., Res. Bul.* No. 232.
8. Sun, T. P. 1932. Histo-physiogenesis of the glands of internal secretion-thyroid, adrenal, parathyroid, and thymus of the chicken embryo. *Physiol. Zoöl.*, **5**, 384-396.

Growth, 1943, **7**, 273-278.

TOLERANCE OF LARVAE OF DROSOPHILA FOR NUCLEIC ACID COMPONENTS: GUANINE*

LOUISE PALMER WILSON

Wellesley College, Wellesley, Massachusetts, and the Marine Experimental Station of the Lankenau Hospital Research Institute, North Truro, Massachusetts
(Received for publication on May 20, 1943)

Since the protein requirements of Drosophila are not known, no mixture of components has been found which will sustain life. It is difficult, therefore, to devise experiments aimed at determining the rôle of protein constituents in normal growth of the animal. This report is one of a series of experiments in which a pure nucleic acid derivative is added to an inadequate diet in order to determine the tolerance of the larvae of Drosophila for the compound in question. With sufficient evidence of this sort it will be possible to derive a mixture of amino acids and nucleic acid derivatives, capable of satisfying the protein requirements. In addition it is hoped that these studies will furnish clues that may lead to the elucidation of the rôle played by each component in normal growth and metamorphosis of the fly.

Yeast, the natural food of Drosophila, imagoes, and larvae, contains four mononucleotides in its complex nucleic acid molecule. Each nucleotide in addition to phosphoric acid and a sugar contains a base, of which two are purine bases, adenine and guanine, and two pyrimidine bases, cytosine and uracil. The tolerance of larvae for adenine has been investigated (Wilson, 1941).

It is the purpose of this investigation to determine the highest concentration of guanine in which normal growth may be maintained. Increase in length and duration of instars in wild type[1] larvae are used as indices of normal growth. Since tests and controls are run simultaneously, any deviation from the normal may be considered as an effect produced by guanine.

Details of experimental procedure and description of culture methods

*Aided by a grant from The Woman's Auxiliary of the Lankenau Hospital Research Institute for cancer research.

[1]Swedish-b wild type Drosophila melanogaster.

are found elsewhere (Wilson, 1941, 1942). Guanine is relatively insoluble in the normal acid culture medium so that saturated solutions or suspensions were used for the experiments. Preliminary investigation showed that saturated solutions had no detectable effect on growth processes of the larvae, consequently suspensions in the salt mixture were used. For the test, 10 mg. of guanine were added to 100 ml. of culture media so that minute particles of guanine were present in each culture dish. Since guanine is slightly more soluble in alkaline medium, a salt mixture with a pH of 7.5, obtained by addition of 0.1 N. NaOH, was used for a series of experiments.

Three experiments using acid medium were performed and three using an alkaline salt mixture. Tests and controls, run simultaneously, were kept in an incubator at 25±1° C. Larvae were killed and measured at 48, 76, 96, and 120 hours after the median hour of oviposition. In each experiment three tubes from the test and three from the control were observed for pupation and emergence of imagoes.

Discussion and Results

In Table 1 are shown the average results for the experiments in which larvae were exposed to a suspension of guanine in acid culture medium. The length in millimeters for larvae killed at the stated intervals after oviposition is shown as well as the number of larvae in each instar. Records of pupae are also included. It can readily be

TABLE 1

Average Length in Millimeters of Larvae Treated with a Suspension of Guanine in Acid Culture Medium

P-pupae. Age in hours from median hour of oviposition. Averages of three experiments.

Age	Control Instar	Control Number	Control Length	Test Instar	Test Number	Test Length
48	1	116	1.66	1	81	1.66
	2	65	2.21	2	57	2.04
76	2	136	3.15	2	113	3.11
	3	19	4.12	3	26	4.16
96	2	5	2.84	2	8	2.36
	3	167	4.90	3	147	4.89
120	3	118	5.40	3	115	5.36
	P	12		P	0	
144	3	2		3	7	
	P	122		P	88	

seen that there is no significant difference between test and control. It seems logical to assume that either the concentration of guanine in solution was too slight to affect length growth and puparium formation in either direction or that guanine is ineffective on larval growth of Drosophila.

To test this, a suspension of guanine in alkaline culture medium was tried. The flies do not exist in nature under alkaline conditions and consequently seem to survive less well in the laboratory in such environment. In addition contamination with bacteria is much more common so that more culture dishes are discarded. There are consequently fewer individuals involved in the data presented in Table 2.

TABLE 2

AVERAGE LENGTH IN MILLIMETERS OF LARVAE TREATED WITH A SUSPENSION OF GUANINE IN ALKALINE CULTURE MEDIUM

P-pupae. Age in hours from median hour of oviposition. Data from a single experiment.

Age	Control Instar	Control Number	Control Length	Test Instar	Test Number	Test Length
48	1	18	1.61	1	18	1.56
	2	10	2.18	2	21	2.08
76	2	24	2.70	2	14	2.92
	3	19	4.27	3	39	3.88
96	2	11	2.59	2	2	3.22
	3	28	4.87	3	35	4.50
120	3	41	5.30	3	31	5.15
	P	1		P	23	
136	3	14		3	4	
	P	21		P	30	
140	3	6		3	0	
	P	23		P	41	
144	P	28		P	33	

It was found nevertheless that growth is significantly slower in the tests.

Upon plotting this data (Figure 1) it was found that the retardation of length growth is not associated with slower moulting. In Table 3 are shown the percentage of individuals found in each instar for each of the three experiments. It will be noted that there is no retardation of moulting or puparium formation from one larval instar to the next. In fact for the most part, moulting and puparium formation occurs slightly earlier in the tests despite the smaller size.

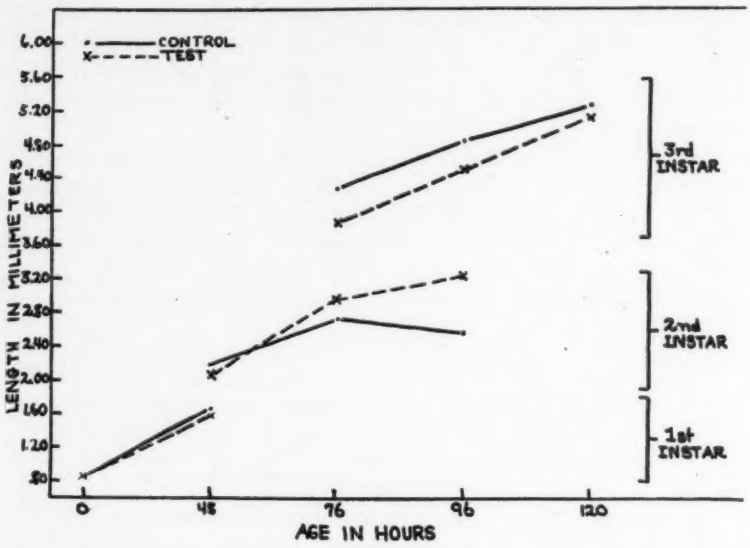

FIGURE 1

RATE OF GROWTH FOR LARVAE TREATED WITH A SUSPENSION OF GUANINE

A similar case of early moulting of short individuals was found when proline was added to acid culture media (Wilson, 1940). In this instance it was suggested that differentiation had been accelerated, for, Hammett and Collings had found that proline furthers the process of differentiation. Later, Hammett (1938) found that initiation and organization in the Obelia hydranth was accelerated by guanine. In Drosophila it is more difficult to separate growth processes but these data give more than a hint that some developmental process is being affected by guanine.

Recently (Bodenstein, 1943) it was discovered that the moulting and puparium hormone are identical or at least derived from the same organ. It is significant that, here, guanine acts to accelerate both larval moults between instars and puparium formation. It may well be that it is concerned in some way with the differentiation and function of the ring gland.

That guanine is concerned with the general growth processes of

TABLE 3

PERCENTAGES (ROUNDED) OF INDIVIDUALS IN LARVAL INSTARS AND PUPAL STAGE FOR EACH EXPERIMENT IN WHICH THE TEST CONTAINED A SUSPENSION OF GUANINE IN ALKALINE CULTURE MEDIUM

Age	Instar	Experiment 1		Experiment 2		Experiment 3	
		Control	Test	Control	Test	Control	Test
48	1	64	55	71	51	51	83
	2	36	45	29	49	49	17
76	2	55	26	100	97	54	42
	3	45	74		3	46	58
96	2	26	5	14		8	3
	3	74	95	86	100	92	97
120	3	98	57	82	68	80	72
	P	2	43	18	32	20	28
136	3	40	8	6	1	41	36
	P	60	92	94	99	59	64
140	3	21					
	P	79					
144	P	100	100	99	100	100	99

initiation and organization of new growth has been further confirmed by recent work on annelid regeneration (Leibmann, 1942*a* and *b*). Guanine with other metabolites is found in chlorogogue cells of these worms. These cells, contrary to general belief, play a very definite rôle not only in supplying nutritive substances but actually in the initiation and speeding up of the healing of wounds and regeneration of new tissue. Leibmann (1942*b*) suggests that guanine may play a specific rôle in this latter process.

Furthermore, guanine has also been found to be active in producing leucocytosis in mammals (Drury, 1936) which certainly involves some developmental processes. It has been found to be a factor which under certain conditions stimulates growth of *Lactobacillus casei* when used to supplement the basal diet (Feeney and Strong, 1942).

In the present experiment there is tied up in the yeast, used as food, 0.06 gm. of guanine per ml. of medium (Meisenheimer, 1921). Under this condition it is difficult definitely to determine the rôle of added guanine, but certainly there is evidence to support the postulate that guanine, "a naturally occurring tissue component of general distribution has its own specific function in developmental growth" (Hammett, 1938) in Drosophila as in Obelia. The results with the former are

consistent with the interpretation derived from the latter that its function is concerned with the growth activity of organization.

When a truly synthetic protein mixture capable of sustaining normal growth in Drosophila has been developed, then and only then, can we more accurately determine this function.

Summary

A suspension of guanine in standard acid culture media at pH of 3.7 is ineffective in modifying length growth or puparium formation in Drosophila larvae.

A suspension of 10 mg. of guanine in 100 ml. of alkaline culture medium of pH of 7.5 slightly accelerates ecdyses and puparium formation in Drosophila larvae. Apparently maximum length growth was not attained because of the intervention of guanine in some process concerned with moulting. There is evidence to support the postulate that guanine plays some specific rôle in organization.

References

1. Bodenstein, D. 1943. Hormones in Drosophila. *Anat. Rec.*, **84** Suppl., 12.
2. Drury, A. N. 1936. Nucleic acid and its derivatives. *Physiol. Rev.*, **16**, 292-325.
3. Feeney, R. E., & F. M. Strong. 1942. Growth stimulating substances for *Lactobaccillus casei*. *J. Amer. Chem. Soc.*, **64**, 881-884.
4. Hammett, F. S., & W. D. Collings. 1937. Developmental growth and l-proline. *Growth*, **1**, 285-290.
5. Hammett, F. S. 1938. Guanine in developmental growth. *Growth*, **2**, 389-385.
6. Liebmann, E. 1942*a*. The rôle of the chlorogogue in regeneration of *Eisenia foetida* (Sav.). *J. Morph.*, **70**, 151-188.
7. ————. 1942*b*. The coelomocytes of Lumbricidae. *J. Morph.*, **71**, 221-249.
8. Meisenheimer. 1921. Hopper-Seyler's *Ztschr. f. Physiol. Chem.*, **114**, 205.
9. Wilson, L. P. 1941. The influence of l-proline on larval growth in *Drosophila melanogaster*. *Growth*, **5**, 11-18.
10. ————. 1942. Tolerance of larvae of Drosophila for nucleic acid components: Adenine. *Growth*, **6**, 1-6.

Growth, 1943, **7**, 279-285.

TOLERANCE OF LARVAE OF DROSOPHILA FOR NUCLEIC ACID COMPONENTS: URACIL*

LOUISE PALMER WILSON

Wellesley College, Wellesley, Massachusetts, and the Marine Laboratory of the Lankenau Hospital Research Institute, North Truro, Massachusetts
(Received for publication on June 8, 1943)

Little is known concerning the requirements of organisms for hydrolytic products of nucleic acids and practically nothing is known concerning the rôle of the pyrimidine and purine bases in specific growth processes. Yeast, with its high percentage of nucleic acid, serves as the chief source of food for the fly, *Drosophila melanogaster*. This report is the third of a series in which the rôle in *Drosophila* of the products formed by hydrolysis of the nucleic acids of yeast is investigated. That these substances do play some rôle in the growth of the larvae is suggested by the fact that high concentrations of pentose nucleotides have been demonstrated in rapidly dividing tissues of *Drosophila* larvae (Caspersson and Schultz, 1939).

The experiments are complicated by the fact that no mixture of known compounds will sustain life for either larvae or adults of these insects. It has been found that adenine sulphate, when added to an inadequate diet would be tolerated only in a concentration more dilute than M/6,000 for adenine (Wilson, 1942). No conclusions were drawn as to the specific rôle of adenine but when guanine was tested, it was found to further organization or some phase of developmental growth in the larvae (Wilson, 1943).

For the pyrimidine base, uracil, it is known that certain lactic acid bacteria either require uracil for reproduction or their rate of reproduction is greatly increased in its presence (Snell and Mitchell, 1941). Hammet (1936) showed that for *Obelia*, regeneration is held back by uracil and differentiation is retarded. This effect may be contrasted to that of guanine which furthers organization in *Obelia* (Hammett, 1938).

*Aided by a grant from The Woman's Auxiliary of the Lankenau Hospital Research Institute for cancer research.

The present paper is an investigation of the tolerance of inadequately fed larvae of Swedish-b wild type *Drosophila melanogaster* for pure uracil. In addition as always in these investigations it is hoped that a hint as to its specific rôle in developmental growth of the larvae may be obtained. The organization of the experiments is the same as described for previous investigations by Wilson (1941, 1942).

Discussion and Results

Table 1 shows the results obtained when uracil was added to the inadequate diet. Figures 1, 2, 3, and 4 compare growth in length of

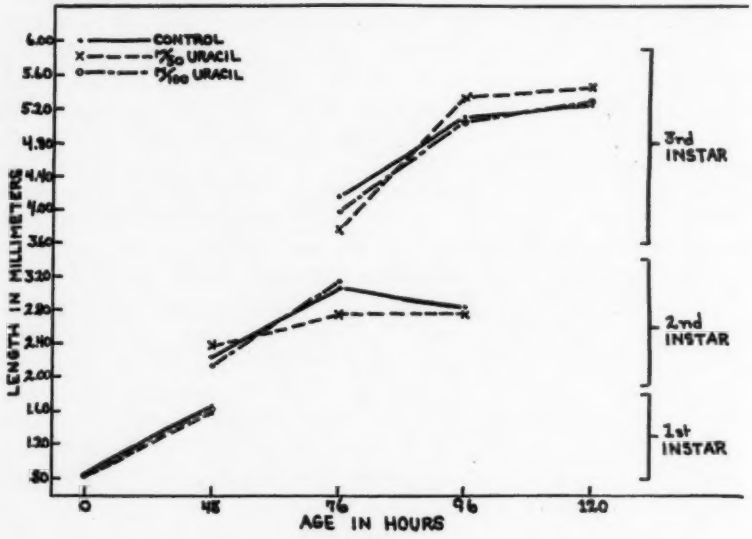

FIGURE 1

(Figures 1-4 show rate of growth of larvae treated with uracil.)

test and control for each instar. A concentration of M/50 which is probably at least 10 times as much as is bound in the yeast supplied as food is not toxic but does slightly retard length in the second instar. In addition there is generally a slower moulting from one instar to the next and a slower eclosion rate. Similar results, however, are obtained in all concentrations down to M/50,000. It therefore seems safe to

TABLE 1

AVERAGE LENGTH IN MILLIMETERS OF LARVAE TREATED WITH URACIL

Age	Ins.	Control			M/50			M/100			M/500			M/1000		
		No.	%	L.	No.	%	L.	No.	%	L.	No.	%	L.	No.	%	L.
48	1	53	63	1.64	32	74	1.60	53	68	1.62	47	46	1.64	52	82	1.61
	2	31	37	2.23	11	26	2.37	24	32	2.12	55	54	2.07	11	18	2.07
76	2	51	57	3.07	20	95	2.74	102	81	3.14	60	81	3.08	53	92	3.12
	3	38	43	4.15	1	5	3.75	22	19	3.98	14	19	4.15	4	8	4.33
96	2	2	1	2.85	6	12	2.76							1	1	3.38
	3	110	99	5.10	47	88	5.34	73	100	5.06	100	100	5.20	77	99	4.82
120	3	34	100	5.25	54	100	5.46	61	100	5.26	48	100	5.29	47	100	5.24
240	I		94			86			90			89			90	
		Control			M/5000			M/10,000			M/25,000			M/50,000		
48	1	75	84	1.60	64	87	1.59	33	88	1.59	57	80	1.63	41	91	1.61
	2	14	16	2.34	10	13	2.23	5	12	2.32	14	20	2.31	4	9	2.03
76	2	79	88	3.15	55	92	3.18	25	100	3.23	30	88	3.09	29	94	3.16
	3	10	12	4.13	5	8	4.68				4	12	4.11	2	6	3.97
96	2	4	5	2.79	2	4	2.31	1	3	3.08	3	7	3.00			
	3	64	95	5.08	47	96	4.97	31	97	4.70	36	93	4.66	29	100	4.62
120	3	48	100	5.45	43	100	5.34	26	100	5.16	29	100	5.29	17	100	5.39
240	I		89			100			82			80			84	

L—Length in Millimeters.
I—Imagoes.

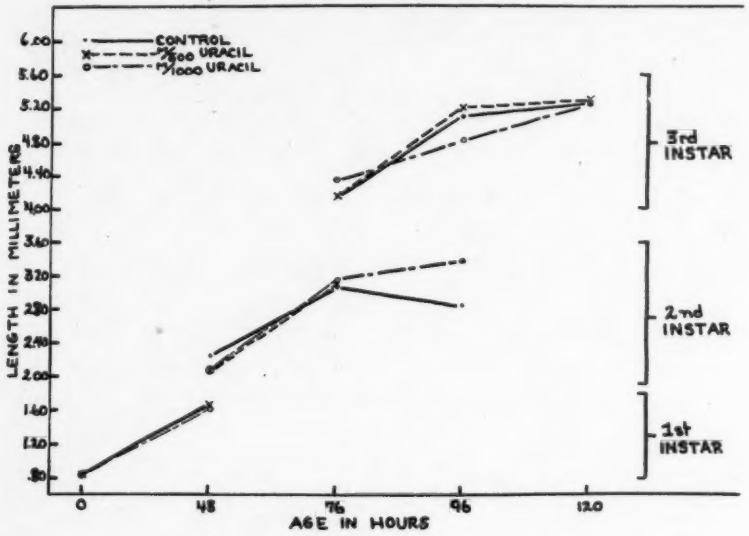

FIGURE 2

assume that we are dealing here with a specific effect of the uracil rather than with any toxic retardation.

The significance of differences in length growth must be evaluated by consideration of the percentage of larvae involved at each measurement. Where the percentage in first and second instars or second and third instars at 48 and 96 hours, respectively, differs greatly the length differences are not so pronounced. This is illustrated in Figures 1 and 2 when compared with percentages shown in Table 1. On the other hand, where percentage differences are less, greater differences in length growth appear as in Figures 3 and 4. These facts are consistent, for the average length differences tend to decrease as more individuals are involved.

It is impossible to tell under conditions of these experiments whether the differences in measurement of length at 120 hours are caused by prepupal shortening or slower growth. Since the lines are straight for growth of third instar test animals, exposed to M/25,000 and M/50,000, it is likely that the tests in these cases are growing slower

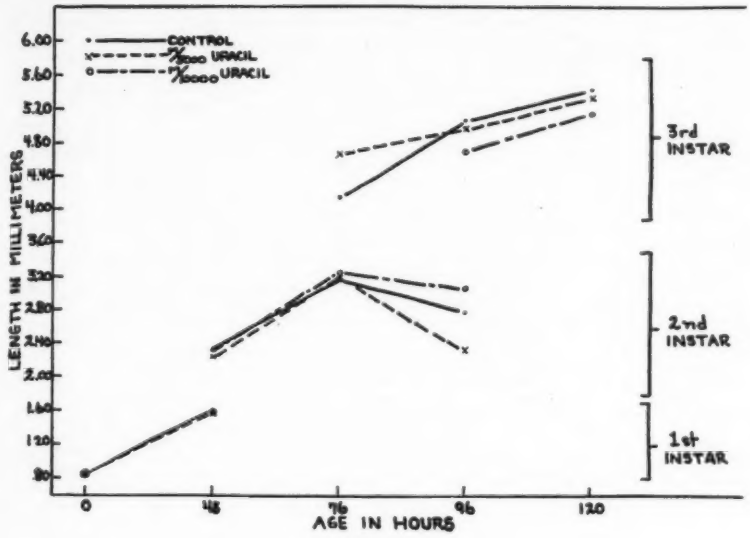

FIGURE 3

than the controls which have already begun to shorten. In the highest concentrations, M/50, M/100, and M/500, where third instar growth is greater than in the control, the results may well be complicated by the fact that uracil is present in sufficient amounts to act as a nutrient. Thus any specific effect on rate of growth may be partially masked.

Recently, Bodenstein (1943) has developed a technique for separating what he calls growth of larval discs from their differentiation. By growth he means cell division and increase in cell size. These latter processes according to him are controlled by a hormone produced in the ring gland and which exerts its effect throughout larval life. When a certain concentration has been attained and a state of increased responsiveness of the organ discs has been reached, this same hormone then controls differentiation. Differentiation is initiated at the time of pupation. If this be true, retarded moulting and slower growth in length such as is reported here might be either an expression of the effect of uracil on the elaboration of the hormone or on the competence of the reacting system. There is no indication whether processes con-

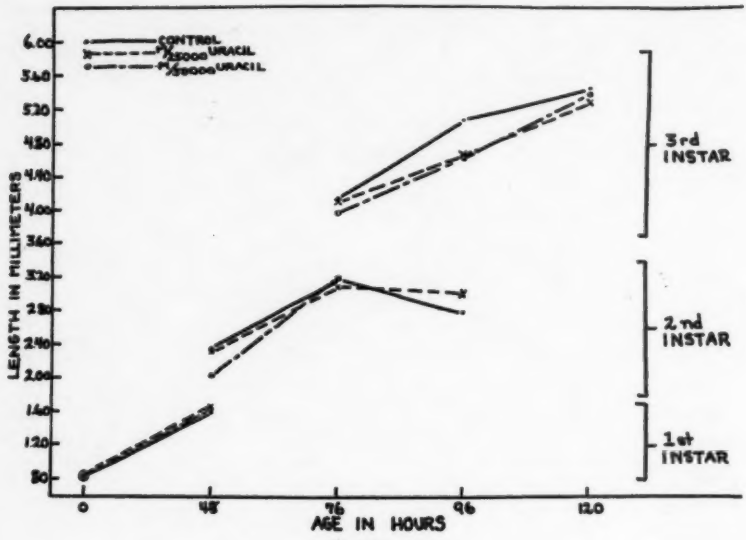

FIGURE 4

cerned with development of adult tissues are involved here. It does seem safe however to assume that uracil plays a specific rôle in larval growth whether it is concerned with the elaboration and expression of the growth hormone or not. Some process concerned with larval developmental growth is retarded in the test animals. Microscopic examination would be necessary to determine whether development of adult organ discs was modified as well.

According to Hammett (1936), differentiation is retarded and proliferation unaffected in Obelia when treated with uracil. One bit of evidence in Drosophila indicates that this may also be the case here. No significant difference in length growth is noted in the first instar for any concentration. There is a slight retardation of moulting into the second instar. Increase in cell size and proliferation are taking place but since no differences in length exist it is evident that uracil plays no rôle in these processes. After the first moult, however, as structures characteristic of the higher instars begin to form, differences appear. In all except the highest concentrations uracil retards the

appearance of these structures. Metamorphosis of the adult organs is also affected, for adults emerge slightly later in most of the tests. This fact should not be emphasized as proof of effect on adult organs, for, late pupation would also retard emergence. Regardless, evidence seems sufficient to warrant the assumption that uracil plays some specific rôle in the larval growth. That rôle is probably the retardation of differentiation.

Summary

Concentrations of uracil as high as M/50 are not toxic to larvae of Drosophila melanogaster.

Uracil depresses differentiation of larvae as measured by duration of larval instars and time of pupation. The differences were slight but consistent between concentrations of M/1000 and M/50,000.

Concentrations of M/50 to M/500 are sufficiently large so as to provide nutriment and thus complicate the growth effects.

References

1. Bodenstein, D. 1943 Hormones and tissue competence in the development of Drosophila. *Biol. Bull.*, **84**, 34-59.
2. Caspersson, T., & J. Schultz. 1939. Pentose nucleotides in the cytoplasm of growing tissues. *Nature*, **143**, 602-603.
3. Hammett, F. S. 1936. Developmental growth and nucleic acid components: I. Uracil. *Biodynamica*, No. 9, 1-12.
4. ————. 1938. Guanine in developmental growth. *Growth*, **2**, 389-395.
5. Snell, E. E., & H. K. Mitchell. 1941. Purine and pyrimidine bases as growth substances for lactic acid bacteria. *Proc. Nat. Acad. Sci.*, **27**, 1-7.
6. Wilson, L. P. 1941. The influence of l-proline on larval growth in Drosophila melanogaster. *Growth*, **5**, 11-18.
7. ————. 1942. Tolerance of larvae of Drosophila for nucleic acid components: Adenine. *Growth*, **6**, 1-6.
8. ————. 1943. Tolerance of larvae of Drosophila for nucleic acid components: Guanine. *Growth*, **7**, 273-278.

Growth, 1943, **7**, 287-290.

TOLERANCE OF LARVAE OF DROSOPHILA FOR NUCLEIC ACID COMPONENTS: CYTOSINE*

LOUISE PALMER WILSON

Wellesley College, Wellesley, Massachusetts, and the Marine Laboratory of the Lankenau Hospital Research Institute, North Truro, Massachusetts
(Received for publication on June 19, 1943)

This report is an extension to cytosine of the general inquiry into the tolerance of Drosophila larvae for derivatives of nucleic acid. The ultimate purpose of such studies is to determine the rôle played in growth by the naturally occurring tissue components. Since a synthetic medium which will permit normal growth has not been devised, it has been the plan of these experiments to test the effect of addition of the substance in question to media in which the amount of yeast is in-

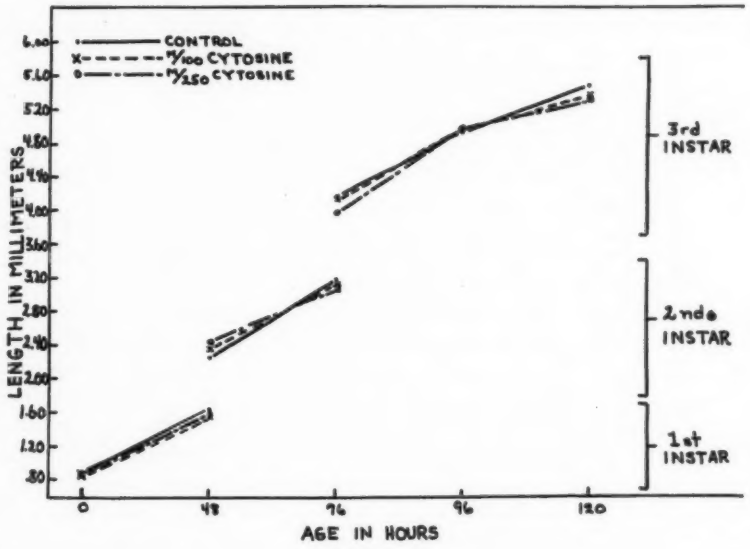

FIGURE 1*a*

FIGURES 1*a* AND 1*b* SHOW RATE OF GROWTH OF LARVAE OF DROSOPHILA TREATED WITH CYTOSINE

*Aided by a Grant from The Women's Auxiliary of The Lankenau Hospital Research Institute for the Promotion of Cancer Research.

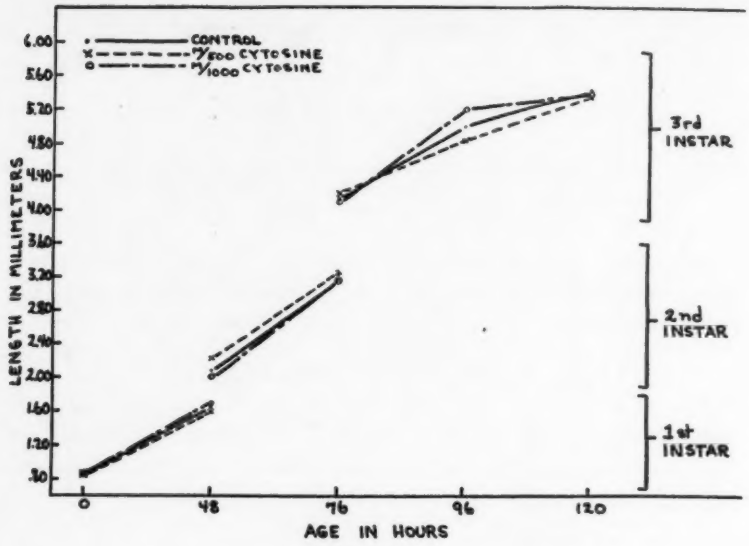

FIGURE 1*b*

adequate for pupation within the normal period. The immediate purpose of this investigation was to determine the concentration of cytosine that larvae of Drosophila will tolerate in their culture medium. It was also hoped that the data might give some indication of the specific rôle of cytosine in growth. The experimental procedure is the same as has been outlined in earlier papers (Wilson, 1941, 1942).

Cytosine in a concentration of M/100 is not toxic to the larvae. This amount is two times as much as required for normal growth, when bound in the yeast. Growth in length and metamorphosis of treated animals is not significantly different from that of the controls. Larvae so treated produce fertile flies upon emergence. Similar results are also obtained in concentrations from M/250 to M/5000. Tables 1, 2, and 3 give average data from the nine experiments performed.

Hammett, Lavine, and Lavine (1937) found that cytosine accelerates differentiation, retards initiation, and slightly increases proliferation in *Obelia*. In certain bacteria cytosine accelerates cell division (Snell and Mitchell, 1941). In these cases the differences are not

TABLE 1

AVERAGE LENGTH IN MILLIMETERS OF LARVAE TREATED WITH CYTOSINE

Age	Ins.	Control		M/100		M/250	
		No.	L.	No.	L.	No.	L.
48	1	129	1.60	90	1.57	88	1.59
	2	9	2.25	17	2.33	18	2.43
76	2	162	3.18	112	3.12	96	3.07
	3	27	4.15	13	4.14	9	3.96
96	3	158	4.92	92	4.95	117	4.95
120	3	133	5.49	110	5.37	98	5.32

TABLE 2

AVERAGE LENGTH IN MILLIMETERS OF LARVAE TREATED WITH CYTOSINE

Age	Ins.	Control		M/500		M/1000	
		No.	L.	No.	L.	No.	L.
48	1	66	1.63	70	1.60	80	1.67
	2	16	2.07	8	2.21	12	2.00
76	2	69	3.15	74	3.24	76	3.15
	3	15	4.15	21	4.20	28	4.10
96	3	97	5.00	94	4.85	102	5.20
120	3	90	5.43	31	5.38	73	5.41
	P			2			

Ins., Instar.
L., Length in millimeters.

TABLE 3

AVERAGE LENGTH IN MILLIMETERS OF LARVAE TREATED WITH CYTOSINE

Age	Instar	Control		M/5000	
		No.	L.	No.	L.
48	1	51	1.60	62	1.63
	2	16	2.10	21	2.07
76	2	71	3.02	68	3.09
	3	19	4.10	22	4.00
96	3	82	5.21	99	5.27
120	3	68	5.49	52	5.32

marked. Rather than assume that cytosine plays an insignificant rôle, it seems more logical to assume that proper criteria for measuring the direct rôle of cytosine have not been discovered. Certainly this ex-

planation seems logical for Drosophila larvae where only growth in length and duration of moulting periods are considered.

It may be concluded then that cytosine is tolerated by Drosophila larvae in concentrations to M/100 and that within the range of M/5000 to M/100 there is no detectible effect on growth, moulting periods, or rate of metamorphosis.

References

1. Hammett, F. S., T. Lavine, & M. Lavine. 1937. Developmental growth and nucleic acid components: V. Cytosine. *Growth*, **1**, 189-193.
2. Snell, E. E., & H. K. Mitchell. 1941. Purine and pyrimidine bases as growth substances for lactic acid bacteria. *Proc. Nat. Acad. Sci.*, **27**, 1-7.
3. Wilson, L. P. 1941. The influence of l-proline on larval growth in Drosophila melanogaster. *Growth*, **5**, 11-18.
4. ———. 1942. Tolerance of larvae of Drosophila for nucleic acid components: Adenine. *Growth*, **6**, 1-6.

Growth, 1943, 7, 291-297.

SULFHYDRYL AND CELL INCREASE IN NUMBER IN THE MYXOMYCETES*

ARTHUR H. BLICKLE

Marine Experimental Station of the Lankenau Hospital Research Institute, North Truro, Massachusetts; and Ohio University, Athens, Ohio
(Received for publication on June 25, 1943)

A. INTRODUCTION

It is well known from the reports of Hammett (1942) and others that cell increase in number is forwarded by sulfhydryl, a naturally occurring chemical group of general distribution. It is important to know whether the nucleus alone may be so stimulated. Long ago Hammett (1933) suggested the use of plasmodium for answer. True it is that Voetglin and Chalkley (1930) had shown increase in nuclear number within single amoebae on exposure to SH, but they also got increase in number of amoebae. So it seemed worth while to see what would happen in plasmodia where nuclear multiplication is the only mark of cell increase in number (1936).

Preliminary to this inquiry, however, some trials were made of the influence of SH on swarm-cell production from vegetative spores of three species of myxomycetes. These were done in order to see if the genus was reactive as genus to the chemical group, and to extend the observations to another group of organisms.

B. SWARM CELL MATERIALS AND METHODS

Spores of freshly collected sporangia of *Stemonitis splendens, Comatricha typhoides,* and *Enteridium roseanum* were transferred to single drops of either tap or distilled water on glass slides, using suction pipettes and sterile needles. The slides were divided into control and test groups of 10 slides each, and placed in large sterile petri dishes lined with moistened filter paper. These were put in an incubator kept at 28° C. The vegetative spores germinated readily in tap-water within 48 hours. At the end of this period the non-motile spores and the

*Aided by a Grant from The Women's Auxiliary of The Lankenau Hospital Research Institute for the Promotion of Cancer Research.

TABLE 1

THE ABSOLUTE AND PERCENTAGE INCREASE OF SWARM CELLS IN CONTROL AND SH-CONTAINING CULTURES OF THREE SPECIES OF MYXOMYCETES

Slide #	At beginning				At end				Calc. No. swarm cells avail. for prolif. (5) (1—3+2)		
	Vegetative cells (1)		Swarm cells (2)		Vegetative cells (3)		Swarm cells (4)				
	Test	Cont.	Test	Cont.	Test	Cont.	Test	Cont.	Test	Cont.	
					Stemonitis Splendens						
1	21	4	37	9	13	2	86	21	45	11	
2	14	17	23	27	9	11	53	38	28	33	
3	11	13	18	21	7	7	32	36	22	27	
4	20	8	36	18	13	5	74	26	43	21	Percentage
5	13	5	14	13	7	3	27	19	20	15	Incr. (4/5)
1a	16	19	51	49	2	1	110	62	65	67	*Test 67%*
2a	7	24	50	61	0	3	79	83	57	82	*Cont. 5%*
3a	2	36	63	54	0	2	122	77	65	88	
4a	8	17	71	47	0	0	134	69	79	64	
5a	12	23	87	67	1	1	156	93	98	89	
Tot.	124	166	450	366	52	35	873	524	522	497	
					Comatricha typhoides						
1	467	431	100	286	265	263	308	401	302	454	
2	642	742	126	378	321	—	472	—	447	—	
3	576	363	204	125	—	141	—	287	—	347	
4	404	435	246	132	336	216	427	313	314	351	Percentage
5	301	680	46	158	72	—	296	—	275	—	Incr. (4/5)
1a	210	109	48	47	42	29	312	197	216	127	*Test 23%*
2a	367	212	144	65	111	145	421	136	400	132	*Cont.—5%*
3a	228	254	47	89	—	201	—	141	—	142	
4a	101	209	17	79	62	—	173	—	56	—	
5a	181	326	39	160	59	216	257	249	161	270	
Tot.	3477	3761	1017	1519	1268	1211	2666	1724	2171	1823	

TABLE 1 (*continued*)

Slide #	At beginning Veget. cells (1)		Swarm cells (2)		Amoeb. cells (3)		At end Veget. cells (4)		Swarm cells (5)		Amoeb. cells (6)		Calc. No. cells—i (7) (1—4+2)		Calc. No. cells—ii (8) (6—3+5)	
	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
								Enteridium roseanum								
1	8	15	28	11	13	19	2	2	203	326	132	100	34	24	322	407
2	4	12	17	19	25	23	3	8	204	286	187	82	18	23	366	345
3	17	17	36	23	47	32	7	14	281	189	141	48	46	26	375	205
4	11	14	21	17	21	21	8	7	246	264	179	44	24	24	404	287
5	7	6	8	9	18	14	1	3	372	367	195	21	14	12	549	374
1a	18	24	27	21	43	56	0	14	—	22	—	91	—	31	—	57
2a	6	14	37	23	76	67	2	5	77	18	311	109	41	32	312	60
3a	13	7	11	44	51	93	0	0	69	26	220	203	24	51	238	136
4a	0	18	14	32	23	45	0	9	45	19	111	114	14	41	133	88
5a	32	11	12	7	101	77	0	2	2	19	285	186	44	16	186	128
Tot.	116	138	211	206	418	447	23	64	1499	1536	1761	992	259	280	2885	2087

Percentage increase (8/7) *Test—1014%; Cont.—645%*

i—Calculated number swarm cells available for proliferation.

ii—Calculated number swarm cells at end.

swarm cells were counted, and the cultures were ready for experiment.

The test cultures were made up to contain 2.6×10^{-7} S as SH in para-thio-cresol. Since this involved the addition of a slight amount of ethanol to the tap-water, an equivalent amount was introduced into the control culture liquid. All precautions noted by Hammett were scrupulously observed. Some experiments were run with 2.6×10^{-8} S as SH; but this concentration was found too low for effectiveness.

After 24 hours incubation the numbers of organisms were re-counted. The counts are tabulated for each of the three experiments together with the percentage increase in swarm cells. It should be especially noted that following germination of the vegetative spores, the swarm cells not only proliferate, but also continue on to develop into the amoeboid state. Other species pass directly into the amoeboid state from the vegetative spores without going through the swarm cell stage.

Every attempt was made to maintain sterile conditions during the 24-hour course of experiment. All apparatus was sterilized at the beginning, and slides were exposed only long enough for inoculation.

C. Swarm Cell Results and Comment

Table 1 gives the counts of vegetative spores, swarm cells, and amoeboid cells obtaining at the beginning and end of culture in control and SH-containing tap-water, together with the percentage increase of the totals for the three species of myxomycetes used in these preliminary trials. Where blanks occur it means that the cultures were contaminated with yeast, protozoa, or bacteria and that such were discarded as unsuitable for decision.

The amoeboid stage was separately considered in the case of *Enteridium roseanum* because this particular organism passes suddenly into this from the swarm cell stage during the 24-hour period of experiment. It will be noted that the calculated number of swarm cells (swarm cells plus amoeboid cells) was greater in the tests than controls at end of experiment even though the number of test swarm cells at beginning before exposure to SH, was less than in the control cultures. It follows that most of the amoebae were derived from dividing swarm cells which proliferated more rapidly under the influence of SH.

The data as a whole indicate that cell increase in number in these myxomycetes is increased by SH as is the case in all other organisms so treated. In other words normal proliferation of these three species

of myxomycetes is stimulated by this naturally occurring chemical group of general distribution. Thus the principle is extended to another genus and can be presumed to be effective therein despite individual idiosyncrasies in proliferation behaviour.

D. Plasmodium Material and Methods

Dormant sclerotia of *Fuligo septica* were extracted from decayed stumps of *Populus alba,* transferred to moist chambers, and incubated at 28° C. for 24 hours during which the plasmodium became active. The specimens were fed powdered oat and/or corn meal. Alternate feedings on macerated sterile filter-paper yielded dirt-free plasmodia.

Anterior fans of small veins were removed from specimens unfed for 24 hours and transferred to sterile micro moist chambers built in hollow-ground slides with circular cover-slips. Two drops of a 2 per cent oat decoction were placed in each chamber. Then to each of five slides was added one drop of para-thio-cresol solution in tap-water equivalent to 2.6×10^{-7} S as SH; and to five others one drop of tap-water containing an amount of ethyl alcohol equivalent to that added to the tests as primary solvent for the reagent. Second drops were added after 24 hours, and at the end of 48 hours the cultures were killed by flooding the cell with 1.5 per cent osmic acid. The specimens were then washed with distilled water and stained in 0.1 per cent aqueous crystal violet, after which they were dehydrated in ascending concentrations of ethanol, transferred to diaphane, and sealed with a cover-slip. The procedure was repeated with *Physarum polycephalum* kindly supplied by Prof. Louise P. Wilson, of Wellesley College. This plasmodium seems to prefer oats to corn, in contrast to *Fuligo* which prefers corn to oats.

Crystal violet gives well stained preparations in which the nuclei may be readily counted. The procedure was to determine the number of nuclei per unit area, using a Leitz microscope carrying a 44 objective and 25 ocular fitted with a standard Leica bacteriological field micrometer. Owing to differences in thickness of the material, differences in distribution of the nuclei, and impossibility of making nuclear counts on the cultured material at beginning of experiment, the data obtained are only crude estimates.

TABLE 2

THE NUCLEAR COUNTS IN UNIT AREAS OF CONTROL AND SH-EXPOSED SAMPLES OF MYXOMYCETE PLASMODIUM FROM *Fuligo septica* AND *Physarum polycephalum*

Fuligo		Physarum	
Test	Control	Test	Control
614	547	1021	943
672	603	1134	1174
692	611	1269	1258
711	697	1306	1365
789	721	1483	1472

E. PLASMODIUM RESULTS AND COMMENT

Table 2 gives the number of nuclei counted in equal areas of test and control specimens in the two sets of trials in order of ascending distribution.

The data show clearly that more nuclei were present per unit area in the SH-exposed *Fuligo* cultures than in the controls. Moreover there was clumping of large numbers of nuclei in the test plasmodia with no indication of a like phenomenon in the controls. The combined findings when taken together with all that has been previously done would seem to allow the conclusion that nuclear increase in number has here been forwarded by SH. On the other hand the fewness of experiments and the crudeness of the method of estimate make the conclusion subject to reservations until better procedure and more experiments can be had.

The only evidence of like action in *Physarum* plasmodia was the clumping of nuclei in the SH cultures. In both species this can be taken as a result of intense localized nuclear proliferation with no concomitant increase in cytoplasmic substance sufficient to effect more uniform distribution. The nuclei of *Fuligo* were larger than those of *Physarum* and more easily counted. In fact those of the latter were so small that there was no real certainty as to their number.

Taking these results at their face value they are seen to be consistent with the finding of Voegtlin and Chalkley (1930) that nuclear increase may be produced by SH; and with the findings of Hammett and Lavine (1940) and Hammett and Wilson (1943) that the nucleus is a locus of specific reaction to this chemical group. From all of which it may be inferred that the nucleus alone may be stimulated by increase in number by SH.

F. Summary and Conclusion

A series of experiments with vegetative and swarm cells of three species of myxomycetes, viz., *Stemonitis splendens, Comatricha typhoides,* and *Enteridium roseanum,* showed that cell increase in number is here as elsewhere forwarded by sulfhydryl. And trials with plasmodia of *Fuligo septica* and *Physarum polycephalum* suggest that this naturally occurring chemical group of general distribution may forward nuclear increase in number alone, though the latter must be taken with reservations until better procedure can be devised and more experiments run. Regardless of this the data as a whole allow the extension of the SH postulate to yet another genus and to several additional species.

References

1. Hammett, F. S. 1933. The influence of sulfhydryl and its sub-oxidized derivatives on the developmental cycle of Hydranths of the genus Obelia. *Protoplasma,* **19**, 510.
2. ———. 1936. The Nature of Growth. Lancaster, Pa.; Science Press.
3. ———. 1942. The rôle of the amino acids and nucleic acid components in developmental growth: I. The growth of an Obelia hydranth. *Growth,* **6**, 59.
4. Hammett, F. S., & T. F. Lavine. 1940. Further localization of SH-action on cell multiplication. *Growth,* **4**, 337.
5. Hammett, F. S., & L. P. Wilson. 1943. The nuclear reaction to SH of excised salivary gland cells of second and early third instar larvae of *Drosophila Melanogaster. Growth,* **7**, 183.
6. Voegtlin, C., & H. W. Chalkley. 1930. The effect of glutathione on cell division in *Amoeba proteus. U. S. Publ. Health Report,* **45**, 3041.

Also:

1. Bary, A. de. 1864. Die Mycetozoen (Schleimpilze). Ein Beitrag zur Kenntnis der Niedersten Organismen. Leipzig.
2. Cook, W. R. I., & E. M. Holt. 1928. Some observations on the germination of spores of some species of Mycetozoa, *Mycologia,* **20**, 340.
3. Lister, A. 1922. A monograph of the Mycetozoa. London. 1894. (2 ed., rev. by G. Lister; 3 ed., 1911.)
4. Macbride, T. H., & G. W. Martin. 1934. The Myxomycetes. New York.
5. Smart, R. F. 1937. Influence of certain external factors on spore germination in the Myxomycetes. *Amer. J. Bot.,* **24**, 145.

Growth, 1943, **7**, 299-307.

ORGAN AND GLAND WEIGHTS OF THE ENGLISH SPARROW

DANIEL P. QUIRING AND PAUL H. BADE

The Cleveland Clinic Foundation; and Western Reserve University, Cleveland, Ohio
(Received for publication on July 10, 1943)

In view of the dearth of data concerning gland and organ weights of the English sparrow (Passer domesticus domesticus), an accurate survey of the weights of the organs and glands of a representative number of adult birds was considered worthwhile. For this purpose 101 birds were captured and killed within 1 to 4 days. Fifty-seven males and 44 females were employed, and weight means were established upon these numbers. Organs were weighed on a chemical balance sensitive to 0.1 milligram immediately after killing the birds with chloroform. Prior to weighing, organs were placed in physiologic salt solution. Excess moisture was removed with a towel.

Since birds were taken between June 25 and February 9, during the period of low sexual activity, and the number of individuals in any given month varied from 3 to 45, no attempt was made to correlate gland size with season. Only five specimens showed sexually active gonads, and the weights of these glands are not included in the data.

Table 1 includes mean weights, standard deviation, and coefficient of variability of body and organs for combined as well as separate sexes.

BODY WEIGHT

The range in body weight for the total group was 14.60 grams, varying from 15.20 to 29.80 grams. Mean body weight for the entire group was 24.17 ± 0.29 grams (Table 1). Mean body weight of 57 males was 24.81 ± 0.35 grams, compared to 23.41 ± 0.47 grams for 44 females. Table 2 shows this difference to be statistically significant.

Tigerstedt (1910) records a body weight of 22 grams for the English sparrow. He does not state the number used. Krohn (1915) found the weight to vary between 27.5 grams and 30.0 grams. The number involved in this series is not mentioned. Wallin (1935, unpublished) shows the following seasonal variations: In a Spring sample of 11 birds,

TABLE 1
MEANS OF ORGAN AND GLAND WEIGHTS

Part weighed	Sex	No.	Mean (Grams)	Standard deviation (Grams)	Coefficient of variation (%)
Body	M & F	101	24.17±0.29	2.947±0.20	12.19±0.88
	M	57	24.81±0.35	2.673±0.25	10.77±1.01
	F	44	23.41±0.47	3.096±0.33	13.22±1.41
Brain	M & F	101	1.014±0.007	0.079±0.006	7.78±0.55
	M	57	1.023±0.008	0.066±0.006	6.44±0.60
	F	44	0.998±0.001	0.075±0.008	7.51±0.80
Thyroid	M & F	100	0.0039±0.0001	0.0006±0.0001	15.38±1.08
	M	56	0.0040±0.0002	0.0012±0.0001	30.00±2.83
	F	44	0.0038±0.0002	0.0015±0.0002	39.47±4.21
Adrenal	M & F	101	0.0059±0.0001	0.0013±0.0001	22.03±1.55
	M	57	0.0060±0.0002	0.0014±0.0001	23.33±2.19
	F	44	0.0058±0.0002	0.0012±0.0001	20.69±2.21
Eyes	M & F	100	0.499±0.005	0.047±0.003	9.40±0.66
	M	56	0.488±0.007	0.053±0.005	10.85±1.02
	F	44	0.507±0.006	0.042±0.004	8.28±0.88
Heart	M & F	101	0.385±0.007	0.071±0.005	18.41±1.29
	M	57	0.400±0.009	0.068±0.006	17.01±1.59
	F	44	0.376±0.011	0.073±0.008	19.39±2.07
Digestive tract	M & F	101	2.602±0.050	0.501±0.035	19.25±1.35
	M	57	2.580±0.056	0.426±0.040	16.52±1.55
	F	44	2.637±0.087	0.576±0.061	21.84±2.33
Kidney	M & F	101	0.338±0.006	0.064±0.045	18.95±1.33
	M	57	0.329±0.008	0.062±0.006	18.81±1.76
	F	44	0.348±0.009	0.063±0.007	18.20±1.94
Liver	M & F	101	1.104±0.021	0.210±0.015	19.02±1.33
	M	57	1.147±0.026	0.196±0.018	17.09±1.59
	F	44	1.048±0.032	0.215±0.023	20.52±2.19
Lung	M & F	101	0.399±0.010	0.102±0.007	25.57±1.94
	M	57	0.407±0.014	0.106±0.010	26.03±2.46
	F	44	0.393±0.015	0.098±0.010	24.95±2.66
Pancreas	M & F	100	0.158±0.003	0.0349±0.002	22.13±1.56
	M	57	0.157±0.005	0.0349±0.003	22.33±2.10
	F	43	0.159±0.005	0.0355±0.004	22.58±2.43
Spleen	M & F	101	0.042±0.003	0.027±0.002	64.61±4.55
	M	57	0.041±0.003	0.026±0.002	62.80±5.89
	F	44	0.042±0.004	0.027±0.003	66.19±7.06
Gonads	M	52	0.0023±0.0004	0.003±0.0003	83.33±8.18
	F	42	0.0059±0.0006	0.004±0.0004	67.79±7.05

the average body weight was 26.8 grams; average body weight of 11 birds trapped in Autumn was 24.8 grams, and of 13 taken in Winter was 26.9 grams.

Miller (1939) states that the average body weight of the 132 birds used in her study was 26.6 grams, or 16 per cent higher than the value of 23 grams obtained by Benedict and Fox (1933).

Other workers have found variable weights. Hrdlicka (1905) records 23.1 grams as average weight of the species. Quiring (1935, unpublished) records an average body weight of 23.51 grams for 26 females and 24.85 grams for 18 males of a series of 44 birds.

Body weight varies greatly, not only from season to season but also over a given 24-hour period. This has been demonstrated by Baldwin and Kendeigh (1938), who found that loss in weight during the night may reach 10 per cent of the total body weight. Besides absence of light and season, age and time of day influence body weight. The amount of food in the intestinal tract is an obviously important factor affecting body weight. Baldwin and Kendeigh show that the contents of stomach and intestines may approximate 5 per cent of the total weight. They point out a daily weight cycle range of about 12 per cent for the English sparrow. Stevenson (1933) concludes that the amount of food in the stomachs of song sparrows, English sparrows, starlings, and white-breasted nuthatches averaged 1.5 per cent of the body weight.

Brain Weight

The lowest brain weight was 0.852 and the highest 1.215 grams, with a range of 0.363 grams. Mean weight for the total group was 1.014 ± 0.007 grams. Mean weight for the 57 males was 1.023 ± 0.008 grams; for 44 females it was 0.998 ± 0.001 grams (Table 1). Actual difference between these means was 0.026 grams, which appears significant (Table 2). Correlation of brain weight with body weight was $+0.128 \pm 0.066$ (Table 3).

The brain weight of 44 birds dissected and weighed by Quiring (1935) was 1.05 grams for 26 females and 1.04 grams for 18 males. In each sex the ratio of brain weight to body weight was 1:23. Hrdlicka (1905) found that the brain weight average was 1.05 grams and that the ratio of brain to body weight was 1:22.

Hrdlicka found that the goldfinch with a body weight of 11.76 grams

TABLE 2

SEX DIFFERENCES IN ORGAN AND GLAND WEIGHTS

Part weighed	57 Males	44 Females	Difference	*SE* of difference	Significance
Body	24.81	23.41	1.40	0.58	S
Brain	1.023	0.998	0.025	0.008	S
Thyroid	0.0040	0.0038	0.0002	0.0002	NS
Adrenal	0.0060	0.0058	0.0002	0.0002	NS
Eye	0.488	0.507	0.019	0.003	S
Heart	0.400	0.376	0.024	0.014	NS
Digestive tract	2.580	2.637	0.057	0.104	NS
Kidney	0.329	0.348	0.019	0.012	NS
Liver	1.147	1.048	0.001	0.041	S
Lung	0.407	0.393	0.014	0.020	NS
Pancreas	0.157	0.159	0.002	0.007	NS
Spleen	0.041	0.042	0.001	0.005	NS

TABLE 3

CORRELATION OF ORGANS AND GLANDS WITH BODY WEIGHT

	No. of birds	r to body weight
Brain	101	$+0.128\pm0.066$
Thyroid	100	$+0.228\pm0.002$
Adrenal	101	$+0.189\pm0.002$
Heart	101	$+0.805\pm0.023$
Liver	101	$+0.451\pm0.044$

had a body to brain weight ratio of 1:19. Quiring (1935, unpublished) tabulates the following ratios: for a phoebe weighing 18.06 grams the ratio was 1:23, for a swallow weighing 18.0 grams 1:20, for a song sparrow weighing 21.0 grams 1:19, and for a bluebird of 29.0 grams 1:21.

In our study of 101 birds, a larger sample than any found in the literature, the brain-body weight ratio for the total group was 1:23, for males 1:24, and for females 1:23.

THYROID GLAND WEIGHT

Mean weight of the thyroid glands for the entire group was 0.0039 ± 0.0001 grams. The smallest thyroid weighed 0.0018 grams and the heaviest 0.0088 grams. The range was 0.0070. The mean weight for males was 0.0040 ± 0.0002 grams compared to 0.0038 ± 0.0002 grams for females. This difference does not appear significant. A correlation of $+0.228\pm0.002$ appeared between thyroid gland and body weight (Table 3).

No values of the English sparrow thyroid weight were found in the literature. Quiring (1935, unpublished) found that the thyroid gland weight average for 44 sparrows was 0.0042 grams. Thyroid-body weight ratios for these and several other birds were as follows: phoebe 1:3010, swallow 1:3583, song sparrow 1:4667, and English sparrow 1:5798.

For 100 of the birds used in this study, the thyroid-body weight ratio was 1:6198. For 56 males the ratio was 1:6202, for 44 females it was 1:6157.

Adrenal Gland Weight

Mean adrenal gland weight was 0.0059 ± 0.0001 grams for the combined group. For the 57 males the mean was 0.0060 ± 0.0002 grams, and for the 44 females it was 0.0058 ± 0.0002 grams (Table 1). Correlation with body weight was $+0.189 \pm 0.002$ (Table 3).

No published data on the weight of the adrenal glands were found. Unpublished data of Quiring (1935) indicate mean weight values of 0.0088 grams for 26 females and 0.0072 grams for 18 male sparrows, taken in Summer. His average for 44 birds was 0.0081 grams. The ratio of adrenal to body weight for these birds was 1:2988, for 18 males 1:3451, and for 26 females 1:2671, compared to our ratio of 1:4096 for the group, 1:4135 for males and 1:4034 for females.

Heart Weight

The combined male and female mean heart weight was 0.385 ± 0.007 grams (Table 1). The lowest heart weight was 0.158 grams, the greatest 0.603 grams. The difference of 0.0233 grams between the means of the sexes appears insignificant (Table 2).

A heart-body weight correlation of $+0.805 \pm 0.023$ appeared. This was the highest correlation observed between any organ and body weights (Table 3).

Parrot, according to Groebbels (1932), determined the heart weight in English sparrows weighing 30 grams at 16.22 grams per kilogram of body weight. He also cites a value of 14.1 grams of heart per kilogram for English sparrows of 25.8 gram body weight obtained by Buchanan. Buchanan also determined a heart weight of 13.2 grams per kilogram for birds weighing 21.0 grams.

Groebbels (1932) records two studies made by Hesse. The first,

made in Germany, was based upon 15 males weighing 29.98 grams with heart weight equivalent to 13.53 grams per kilogram, and 10 females averaging 28.17 grams per kilogram. The second study was made in St. Petersburg (Leningrad) on eight males, average body weight 33.44 grams, and five females, average body weight 31.92 grams. The heart weight for the males was 15.52 grams and for the females 14.84 grams per kilogram of body weight.

In our study the heart weight was 15.94 grams per kilogram of body weight.

Magnan (1911) found that average heart weight for passerine birds varied from 12.5 to 13.3 grams per kilogram of body weight.

Liver Weight

Individual liver weights ranged from 0.707 to 1.564 grams. The mean was 1.104 ± 0.021 grams (Table 1). The actual difference of 0.099 grams between the mean liver weights of males and females appears statistically significant (Table 2). A liver-body weight correlation of $+0.451 \pm 0.044$ existed (Table 3).

The mean liver weight was 45.6 grams per kilogram of body, and the liver-body ratio was 1:21. Magnan (1911) states that liver in passerine birds was approximately 34.1 grams per kilogram of body weight. Our value is not in conflict with that of Magnan, as larger passerine birds were found by Headley (1895) to have relatively smaller liver weights than smaller members. Headley shows that the swallow has a liver-body weight ratio of 1:18. He lists the following ratios for larger birds: tern 1:20, common fowl 1:26, and tawney owl 1:43.

Kidney Weight

Kidneys ranged in weight from 0.198 to 0.473 grams. Mean weight for the total group was 0.338 ± 0.006 grams. The value for the male was 0.329 ± 0.008 grams. Mean weight for the female kidney was 0.348 ± 0.009 grams (Table 1). The difference between the means, 0.019 grams, is insignificant (Table 2).

Magnan found a value of 12.7 grams of kidney per kilogram of body weight for the passerine birds. For the English sparrow we found a value of 13.9 grams per kilogram of body.

Digestive Tract Weight

Digestive tract weight ranged from 1.538 to 3.816 grams. Mean weight for the group was 2.602±0.050 grams; for males it was 2.580±0.056 and for females 2.637±0.087 grams (Table 1). The difference of 0.057 grams between the sexes appears insignificant (Table 2).

Groebbels (1932) concludes that avian viscera represent approximately 41 per cent of the total body weight. We did not weigh visceral tract and organs intact, but the total weight of visceral organs, obtained by addition, averaged 6.55 grams, or 27 per cent of the total body weight. Groebbels does not indicate the species used in his determination, or whether the intestinal tract was empty or filled.

Lung Weight

Lungs varied from 0.237 to 0.738 grams in weight. The value for the males was 0.407±0.014 grams and for females 0.393±0.015 grams; the difference appears insignificant.

Pancreas Weight

Pancreas weights ranged from 0.082 to 0.255 grams. The mean weight for 100 specimens was 0.158±0.003 grams (Table 1). A weight difference of 0.003 between the sexes appears insignificant (Table 2).

Spleen Weight

The spleen weight ranged from 0.006 to 0.146 grams, the greatest range observed for any organ. The mean weight for the group was 0.042±0.003 grams, for the males 0.041±0.003 and for the females 0.042±0.004 grams; the difference is not significant (Tables 1 and 2).

For sparrow lung, pancreas, and spleen published weight data appear to be lacking. We found the lung-body weight ratio to be 1:60, pancreas 1:152, and spleen 1:576.

Gonad Weights

The testes, apparently quiescent at the time the birds were killed, had a weight range of 0.0009 to 0.0127 grams. Most of the weights were between 0.0009 and 0.0039 grams. Mean testes weight for 52 male birds was 0.0023±0.0004 grams. In four birds the testes were

definitely active, and these weighed 0.0159, 0.0304, 0.0960, and 0.3460 grams. They were not included in these calculations.

Ovaries ranged from 0.0012 to 0.0123 grams. Mean weight for this organ was 0.0059±0.0006 grams (Table 1). Ovaries of two females appeared active and their weights, 0.0217 and 0.0279 grams, were disregarded.

Our values for the testes and for the ovary cannot fairly be compared to those of other workers unless birds were taken in the same period of low sexual activity. Range in testes weight from 0.0020 to almost 0.4000 grams recorded by Wallin (1935, unpublished) shows how easily conflicting values arise.

Eye Weight

Eye weights ranged from 0.411 to 0.638 grams. Mean weight for the total group was 0.499±0.005 grams (Table 1). For females it was 0.507±0.006 grams, exceeding that of males by 0.019 grams (Table 2).

Headley (1895) determined the ratio of eye to body weight in several large birds. Among the ratios were owl 1:33, falcon 1:35, woodpecker 1:73, peacock 1:326, and goose 1:567. The ratio of eye to body weight in our study is 1:47.

Summary

1. One hundred and one English sparrows were weighed and dissected, and the weights of individual organs and glands were determined. Mean body weight as well as mean weight for each organ and gland was then established.

2. The weights of the body, brain, and liver, statistically treated, were significantly higher in the males. Eye weight was significantly higher in females. Thyroid, adrenal, heart, digestive tract, kidney, lung, pancreas, and spleen weights showed no significant difference between the sexes.

3. Correlation between organ weights and body weight showed the following values: heart +0.805±0.023, liver +0.451±0.044, brain +0.128±0.066, thyroid +0.228±0.002, and adrenal gland +0.189 ±0.002. With the exception of heart and liver, the degree of correlation was low.

REFERENCES

1. BALDWIN, S., & S. C. KENDEIGH. 1938. Variations in the weight of birds. *Auk*, **55**, 416-467.
2. GROEBBELS, F. 1932. Der Vogel. Berlin: Gebrüder Borntraeger.
3. HEADLEY, F. W. 1895. Structure and Life of Birds. New York: Macmillan.
4. HRDLICKA, A. 1905. Brain Weight in Vertebrates. *Smithsonian Misc. Collect.*, **48**, 101 (June 10).
5. MAGNAN, A. 1911. *Bull. Museum Hist. Nat., Paris*, **17**, 492-493, 581.
6. MILLER, D. S. 1939. A study of the physiology of the sparrow thyroid. *J. Exper. Zool.*, **80**, 259-285 (July).
7. QUIRING, D. P. 1935. Unpublished data on small birds. Cleveland Clinic Foundation.
8. STEVENSON, J. 1933. Experiments on the digestion of food by birds. *Wilson Bull.*, **45**, 155-167.
9. TIGERSTEDT, R. 1910. Die Produktion von Wärme und der Wärmehaushalt. *Winterstein's Handb. der Vergleich. Physiol.*, Vol. 3.
10. WALLIN, H. E. 1935. Normal seasonal variations in the gonads and thyroids of the English sparrow. (Unpublished, Western Reserve University.)

Growth, 1943, **7**, 309–315.

METABOLISM OF THE ENGLISH SPARROW

DANIEL P. QUIRING AND PAUL H. BADE

The Cleveland Clinic Foundation; and Western Reserve University, Cleveland, Ohio
(Received for publication on July 10, 1943)

In connection with establishing body and gland weights of the English sparrow (Passer domesticus domesticus), shown in Table 1, during the period of low sexual activity (June 25 to February 1), basal metabolic rates were determined as well. For this purpose 15 males and 15 females were selected from the number trapped. In 131 tests under basal conditions the oxygen used and the carbon dioxide released were determined. The values derived are expressed in cubic centimeters per 24 hours for the combined sexes and for each sex separately in Table 2.

From the data gathered an attempt was made to determine the possible relationship between gland and body weight and metabolism rate in the sparrow (Table 3).

Metabolism tests were made on the 30 individuals after a captivity of two to five days. Each bird was killed within 24 hours after the last metabolism determination and weights were determined immediately.

The apparatus used for the tests was in principle that described by Regnault (1849), modified by Schwabe and Griffiths (1938) and by us. This machine is easily operated, and slight changes in activity of the bird appear on the kymographic record, making it possible to select periods of quiescence for determination of basal data. It is a closed system operating at atmospheric pressure, the carbon dioxide is removed as quickly as formed, and a permanent kymographic record of the oxygen consumed (Figure 1) is written by the bird.

Carbon dioxide was determined by titration of a standard barium hydroxide solution through which exhaled air was drawn. A preliminary test of 30 to 40 minutes enabled the birds to adjust themselves to the chamber. The oxygen values obtained were reduced to standard conditions.

A truly basal record shows a continuous and equal rise per unit of

TABLE 1

MEANS OF BODY AND GLAND WEIGHTS

Part weighed	Sex	No.	Mean (Grams)	Standard deviation (Grams)	Coefficient of variation (%)
Body	M & F	30	23.70±0.44	2.41±0.31	10.14±1.31
	M	15	24.30±0.53	2.07±0.38	8.11±1.48
	F	15	23.03±0.48	1.88±0.34	7.71±1.41
Brain	M & F	30	1.000±0.015	0.083±0.011	8.31±1.07
	M	15	0.997±0.020	0.076±0.014	7.64±1.39
	F	15	1.003±0.023	0.089±0.016	8.88±1.62
Thyroid	M & F	30	0.0041±0.0003	0.0016±0.0002	39.51±5.10
	M	15	0.0036±0.0003	0.0011±0.0002	31.31±6.26
	F	15	0.0047±0.0005	0.0019±0.0003	41.54±7.58
Adrenal	M & F	30	0.0055±0.0002	0.0014±0.0002	24.77±3.19
	M	15	0.0056±0.0004	0.0014±0.0003	25.71±4.69
	F	15	0.0055±0.0003	0.0013±0.0002	23.81±4.35
Eye	M & F	29	0.482±0.006	0.033±0.004	6.98±0.92
	M	15	0.471±0.008	0.031±0.006	6.61±1.21
	F	14	0.493±0.008	0.032±0.006	6.45±1.22
Heart	M & F	30	0.395±0.010	0.054±0.007	13.74±1.77
	M	15	0.397±0.017	0.064±0.012	16.11±2.94
	F	15	0.395±0.011	0.043±0.008	10.93±2.00
Dig. tract	M & F	30	2.420±0.066	0.360±0.046	14.87±1.92
	M	15	2.460±0.073	0.284±0.052	11.54±2.11
	F	15	2.367±0.107	0.416±0.076	17.57±3.21
Kidney	M & F	30	0.299±0.010	0.054±0.007	18.16±2.34
	M	15	0.291±0.013	0.052±0.010	18.04±3.29
	F	15	0.307±0.015	0.058±0.011	18.95±3.46
Liver	M & F	30	0.990±0.038	0.207±0.027	20.90±2.70
	M	15	1.070±0.060	0.232±0.042	21.68±3.96
	F	15	0.917±0.042	0.161±0.029	17.56±3.21
Lung	M & F	28	0.375±0.018	0.095±0.013	25.38±3.39
	M	15	0.373±0.019	0.073±0.013	19.54±3.57
	F	13	0.372±0.021	0.074±0.015	20.00±3.93
Pancreas	M & F	29	0.142±0.006	0.031±0.004	21.83±2.87
	M	15	0.139±0.006	0.025±0.004	17.73±3.24
	F	14	0.146±0.010	0.036±0.007	24.99±4.72
Spleen	M & F	28	0.031±0.002	0.013±0.002	42.48±5.68
	M	13	0.032±0.004	0.015±0.003	48.28±9.55
	F	15	0.029±0.002	0.009±0.002	32.63±5.96
Gonads	M	14	0.003±0.001	0.001±0.001	46.87± 8.86
	F	13	0.007±0.001	0.004±0.001	63.38±12.45

TABLE 2
METABOLISM DATA

	Sex	No.	Mean (Cc.)	Standard deviation (Cc.)	Coefficient of variation (%)
Oxygen per 24 hours	M & F	131	2053±40.3	462±28.68	22.50±1.40
	M	65	2198±54.8	445±39.03	20.24±1.78
	F	66	1911±53.3	435±28.68	22.60±1.99
CO_2 per 24 hours	M & F	129	1762±32.1	365±22.72	23.11±1.44
	M	63	1906±42.1	334±30.65	19.53±1.74
	F	66	1625±43.2	351±30.57	24.07±2.10
Respiratory quotient	M & F	128	0.851±0.007	0.084±0.005	10.99±0.69
	M	63	0.860±0.012	0.095±0.008	12.30±1.10
	F	65	0.854±0.007	0.054±0.005	7.16±0.63
Calories per 24 hours	M & F	135	10.07±0.22	2.58±0.16	25.62±1.56
	M	68	10.90±0.32	2.64±0.23	24.93±2.09
	F	67	9.38±0.27	2.23±0.19	23.60±2.05
Calories per gram brain	M & F	135	10.12±0.23	2.72±0.16	26.67±1.62
	M	68	10.91±0.33	2.72±0.23	24.93±2.15
	F	67	9.32±0.27	2.20±0.19	23.60±2.04
Calories per square centimeter	M & F	135	0.1200±0.0024	0.0284±0.0017	23.66±1.41
	M	68	0.1246±0.0036	0.0304±0.0026	24.39±2.09
	F	67	0.1131±0.0031	0.0261±0.0022	23.07±1.99

TABLE 3
CORRELATION OF GLANDS WITH METABOLISM

	No. birds	Sex	No. tests	r to metabolic rate
Body surface	30	15 M, 15 F	135	+0.416±0.105
Body	30	15 M, 15 F	135	+0.321±0.012
Liver	29	14 M, 15 F	131	+0.269±0.116
Adrenal	30	15 M, 15 F	135	+0.159±0.120
Heart	30	15 M, 15 F	135	+0.131±0.121
Brain	30	15 M, 15 F	135	+0.123±0.023
Thyroid	30	15 M, 15 F	135	—0.321±0.012

time (Figure 1). Deviations from a regular rise indicate activity of the animal or improper functioning of the machine.

From oxygen and carbon dioxide values obtained, the respiratory quotient was calculated.

The birds were kept in an aviary that permitted short flights from time of capture until determinations were made. Temperature was held near thermal neutrality (22° to 24° C.) before and during the tests. Food was removed 12 hours before the test. Absence of intestinal

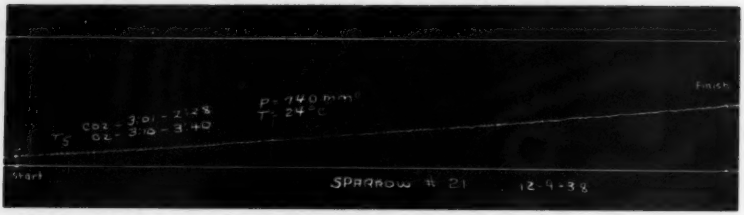

FIGURE 1

KYMOGRAPHIC RECORD OF THE METABOLISM OF THE ENGLISH SPARROW

The apparatus was calibrated so the actual height of the slope of the recorded line made the determination of oxygen consumed for a given interval an easy matter.

contents substantiated the postabsorptive condition. Determinations were made between 2:00 and 4:30 P.M.

One hundred and thirty-one oxygen determinations were made (Table 2). The mean was 2053±40.3 cc. of oxygen consumed per bird per 24 hours. Sixty-five tests were made on males. Mean oxygen consumption was 2198±54.8 cc. per 24 hours.

The remaining 66 tests were made on 15 females. Mean oxygen consumption was 1911±53.3 cc. per 24 hours.

Sixty-three carbon dioxide determinations were made on males. The release of carbon dioxide was 1906±42.1 cc. per 24 hours per bird.

Sixty-six tests on 15 females gave a value of 1625±43.2 cc. per bird per 24 hours.

For the entire group, carbon dioxide release was 1762±32.1 cc. per bird per 24 hours (Table 2).

The respiratory quotient for the group was 0.851±0.007. The quotient for the males exceeded that of the females by 0.018; the difference is not significant (Table 2).

Mean oxygen consumption was 10.07±0.22 Calories per 24 hours (Table 2). Values for the male were 10.90±0.32 Calories and for the female 9.38±0.27 Calories per 24 hours. The difference appears statistically significant. The number of Calories per gram of brain per 24 hours for the group is 10.12±0.23.

The basal metabolic rate of the sparrow expressed as Calories per square centimeter per 24 hours is 0.1200±0.0024, or 1200 Calories per square meter of body surface. For males the rate is 0.1246±0.0036 Calories and for females it is 0.1131±0.0031 Calories. This difference

appears significant. The values of 1200, 1246, and 1131 Calories per square meter of surface area are not far beyond the value of 1000 Calories per square meter on which Rubner attempted to base his surface area metabolism law, but to which, as Benedict pointed out, there are many exceptions (Table 2).

An attempt was made to correlate the weight of the body, brain, thyroid gland, adrenal gland, and liver, and the body surface area with the metabolic rate. The weights of these organs are indicated in Table 1. The values of the attempted correlations are expressed in Table 3.

Surface area had the highest degree of correlation with metabolism, namely $+0.416 \pm 0.105$ in terms of "*r*". Body weight stood next, with an *r* value of $+0.321 \pm 0.012$. The values for the remaining glands and organs stood in the following order: liver $+0.269 \pm 0.116$, adrenal gland $+0.159 \pm 0.120$, heart $+0.131 \pm 0.121$, and brain $+0.123 \pm 0.023$. The thyroid showed a negative correlation -0.321 ± 0.012.

Tigerstedt (1910) was the first to establish definite values for oxygen consumption in the English sparrow. He states that the oxidation rate was 755 Calories per kilogram of body weight per 24 hours. He does not indicate that the birds were under basal conditions. The average body weight of his birds was 22 grams. The number of birds used is not stated. Birds with a body weight of 22 grams have a surface area of 78.51 sq. cc., according to the Benedict-Fox formula of $S = W\ 2/3 \times K$. If the birds used by Tigerstedt had a surface area of 78.51 sq. cm. and an oxidation rate of 755 Calories per kilogram of body weight per 24 hours, the oxidation rate per square meter of body surface would be 2115 Calories. The oxidation rate could also be expressed as 16.61 Calories per bird per 24 hours.

Benedict and Fox (1933) recorded oxygen consumption of 5.2 Calories per bird per 24 hours, or 643 Calories per square meter of body surface, for seven English sparrows.

Miller (1939) determined an oxygen consumption of 789 Calories per square meter of surface for 132 English sparrows. To determine seasonal variation, Miller ran a series of tests on 12 birds in October and found the value to be 2987.7 cc. of oxygen per 24 hours. For eight birds tested in November the oxygen consumption was 3197 cc. per 24 hours. In December the average value for eight birds was 3255 cc. per 24 hours. In January 20 birds showed an average oxygen con-

sumption of 2973 cc. per 24 hours. In February 22 birds averaged 2681 cc. Converting these values to Calories per 24 hours, the averages run as follows: October, 14.33 Calories; November, 14.88 Calories; December, 15.36 Calories; January, 13.92 Calories; and February, 12.48 Calories per bird per 24 hours.

Benedict and Fox (1933) found that after birds fasted for 20 hours, if tested at a temperature of 27.6° C., activity might cause the oxygen consumption to rise to 1439 Calories per square meter, approximately 11.30 Calories per bird per 24 hours. They show further that if the tests were made at 25.8° C. with muscular activity at a minimum, a respiratory quotient of 0.70 to 0.72 existed, and the oxygen consumption dropped to 637 Calories per square meter.

It will be seen that the values of various investigators differ widely. This difference may be attributed to seasonal distribution, difference in stock and nutrient condition, temperature differences, muscular activity or unrest, the point in the daily metabolic cycle at which the tests were made, and the age of the birds tested.

Summary

1. Basal metabolism determinations were made on 30 sparrows, 15 of each sex, at a temperature between 22° and 24° C.

2. The oxygen consumption per bird per 24 hours based on 131 determinations was 2053±40.3 cc. For 15 males the value was 2198±54.8 cc., and for 15 females it was 1911±53.3 cc.

3. The carbon dioxide release for 30 birds in 129 tests was 1762±32.1 cc. per bird per 24 hours. For 15 males the mean, based on 63 determinations, was 1906±42.1 cc. per bird. Sixty-six tests on 15 females gave a mean carbon dioxide release of 1625±43.2 per bird per 24 hours.

4. The respiratory quotient for 30 birds was 0.851±0.007. The values for 15 males and 15 females were 0.860±0.012 and 0.854±0.007 respectively.

5. Metabolism expressed in Calories per 24 hours per bird was 10.07±0.22, a value based on 135 determinations. From 68 determinations made on 15 males the mean was 10.90±0.32 Calories. The mean of 67 tests on 15 females was 9.38±0.27 Calories per bird per 24 hours. The difference between these values appears significant.

6. Metabolism per gram of brain based on 135 tests, using 30 birds,

was 10.12±0.23 Calories. Sixty-eight tests on 15 males gave a mean of 10.91±0.33 Calories per gram of brain per bird. Sixty-seven tests on 15 females gave a mean of 9.32±0.27 Calories per bird per gram of brain.

7. Values of 1200, 1246, and 1131 Calories per square meter of body surface have been established respectively for 30 birds, for 15 males and for 15 females.

8. Varying degrees of correlation occurred between body weight, organ weights, and surface area and basal metabolism. Body surface related to metabolism gave an r value of +0.416±0.105, body weight to metabolism +0.321±0.012, liver to metabolism +0.269±0.116, adrenal gland to metabolism +0.159±0.120, heart to metabolism +0.131±0.121, brain to metabolism +0.123±0.023, and thyroid to metabolism —0.321±0.012.

References

1. Benedict, F. G., & E. L. Fox. 1933. Der Grundumsatz von kleinen Vögelin (Spatzen, Kanarienvögeln und Sittichen). *Arch. f. d. ges. Physiol.*, **232**, 357.
2. Miller, D. S. 1939. A study of the physiology of the sparrow thyroid. *J. Exper. Zoöl.*, **80**, 259 (July).
3. Regnault, N. M., & J Reiset. 1849. Recherches chimiques sur la respiration des animaux des diverses clases. *Ann. de chim. et phys.*, **26**, 299.
4. Schwabe, E. L., & F. R. Griffiths, Jr. 1938. An easily constructed rat metabolism apparatus. *J. Nutrition*, **15** (Feb.).
5. Tigerstedt, R. 1910. Die Produktion von Wärme und der Wärmehaushalt. *Winterstein's Handb. der Vergleich. Physiol.*, **3**.

BOOK REVIEWS

Growth, 1943, **7**, 317.

(*James A. Reyniers, Ed. Micrurgical and Germ-Free Methods. Springfield, Ill.: Thomas, 1943. Pp. 274.*)

REVIEWED BY DALE R. COMAN

This book is a symposium on the application of micrurgical and germ-free techniques to experimental biology and medicine. Contributors to the volume include, in addition to the editor, Philip C. Trexler, M. J. Kopac, E. M. Hildebrand, Oram C. Woolpert, N. Paul Hudson, R. W. Glaser, Philip R. White, I. Rosenstern, F. Kammerling, Wm. F. Wells, and others. The book is the result of a colloquium held at Notre Dame in 1939 upon basic bacteriological techniques. The plan of the participants was to stress technical methods and their applications to various problems such as cross-infection.

Chapter I considers the design of micrurgical machines, including micromanipulators, pipettes, and needles. This subject is covered in considerable detail. In Chapter II the application of micrurgical methods to studies on the surface chemistry of living cells is dealt with by M. J. Kopac. This includes methods for measuring interfacial tensions and adsorption. The application of micrurgy to phytopathological problems is considered in Chapter III. Then follows a section on germ-free methods and the development of germ-free systems, the rearing of germ-free animals of various kinds, and the use of the mammalian fetus, invertebrates, and plants under germ-free conditions. Lastly, the control of cross infection by the use of mechanical barriers in hospitalized infant population is considered. The use of ultraviolet as a barrier is discussed by Wells. The recent work on propylene glycol and ethylene glycol as air disinfecants is not mentioned.

This little volume is well worth reading by anyone working in biological fields since it holds a wealth of material on methods that can be applied in widely diverse types of investigation. The subject matter has been painstakingly presented in a surprising wealth of detail.

Growth, 1943, **7**, 319.

(Philip R. White. A Handbook of Plant Tissue Culture. Lancaster, Pa.: Cattell Press, 1943.)

REVIEWED BY FREDERICK S. HAMMETT

The Lankenau Hospital Research Institute, Marine Biological Laboratory, North Truro, Massachusetts

---

In clean, precise, and always genial English this book gives the essence of plant tissue culture distilled from 457 articles and the extensive experience of the author in this and cognate fields. Without padding, but with all necessary detail we are given the historical background, the materials, the laboratory set-up, the nutrients and their preparation, the techniques of culture, the methods of measurement and record, and the implications of results to plant physiology, pathology, and morphogenesis. The potentialities of such procedures for getting at the fundamentals of cellular activities are indicated. The work is illuminated by well-chosen pictures, diagrams, and tables. Photographs of the leading workers in the field are given. The precautions to be taken and the pit-falls to be avoided are clearly set forth. All in all this little volume will undoubtedly be the vade mecum of all those who till this potentially fruitful field for many years to come.

Growth, 1943, **7**, 321-322.

(Sir d'Arcy Wentworth Thompson. On Growth and Form. New York: Macmillan, 1943. Pp. 1116.)

REVIEWED BY FREDERICK S. HAMMETT

Marine Biological Laboratory of the Lankenau Hospital Research Institute, North Truro, Massachusetts

This book is an intelligent and elegant presentation of how growth and the forms it produces may be expressed in terms of physical forces and mathematical formulae. The assemblage of data is impressive in amount; pleasing in variety; and enticing in novelty. The treatment is masterly; and the virtual refusal to admit other factors than the physical is genial. Urbanity, however, is an insufficient cloak against the clear cold winds of fact. And it is a fact that present knowledge, limited though it surely is, does not allow the interpretation of the facts of life on one set of forces alone, be these physical, chemical, or genetic. Now the progress of living goes on the four wheels of Direction; Substance; State of Substance; and Form. The author has used but two of these for his carryall, viz., State of Substance and Form. If he had used all four he would have undoubtedly produced the materials for integration of the interdependencies of living. His virtual denial that genetic direction and chemical substance and reaction are significant participants in growth and the forms it produces, make his document a restricted analysis, eminent and illuminating though this may be. This is no place to enter into an exhaustive disquisition on the distinction between chemical and physical properties and activities; on how physical phenomena are as much the product of chemical forces as they are the producers thereof; and how neither gravity, surface tension, light, heat, electricity, nor osmosis adequately account for the difference between an elephant and a giraffe, or how it comes about that mice give birth to mice and not to elm trees, and elms produce elms and not mice. This matter of direction of growth and form is not so easily disposed of. It certainly isn't to be disposed of by either the physical principles or the mathematics of engineering as at present known. As far as this reviewer is concerned it seems as if progress towards an integrated understanding of growth as well as of the other

phenomena of living can best be made at present by considering them as resultants of the four interdependent aspects of things; viz., Direction as comprised in the science of Genetics; Substance as comprised in the science of Chemistry; State of Substance as comprised in the science of Physics; and Form as comprised in the science of Anatomy. To exalt one and decry the others seems hardly cricket. The application of the Paretan doctrine of Interdependence, which by the way was recognized in its essentials by the Ancient Hindus long before the Christian era, might well help resolve the confusion arising from our proneness to lock the phenomena of living each into its own little safety deposit box. Despite this stricture the volume deserves to be owned and browsed through by all who desire equilibration. It is an historical document. It consists of 1116 pages, 554 figures, and two beautiful plates of a splash of milk. There is a good index and an uncounted number of references of adequate format. To have included the contributions of Ales Hrdlicka, Wm. E. Castle, H. S. Burr, Paul Weiss, and Wm. Seifriz would have added to its stature. The type gives comfortable reading, and the book is adequately bound to carry its 3 and 3/16 pounds of weight. It is published by the MacMillan Company and the Cambridge University Press. It costs $12.50.